Ring nematode (*Criconemoides xenoplax*), distribution, characterisation and culture methods

by
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ABSTRACT

The ring nematode, *Criconemoides xenoplax* (Raski, 1952) Loof & De Grisse, is described as a migratory ectoparasitic nematode that feeds entirely on the roots of plants, mainly those of woody perennials. The ring nematode is regarded, worldwide, as a significant pest in stone fruit orchards and vineyards, and it has become a common soil pest in South African production areas. To determine the distribution of the ring nematode, a survey was conducted in grapevine and stone fruit production areas, in both the Western and Northern Cape provinces. Nematode specimens collected during the study were characterised morphometrically and molecularly to determine the ring nematode species present in the areas. Soil samples were obtained from randomly selected farms, with additional data acquired from the diagnostic laboratory, Nemlab (Klapmuts, Paarl, Western Cape province), being investigated in relation to ring nematode occurrence in soil samples from the stone fruit and grapevine areas.

After DNA characterisation of the Internal Transcribed Spacer (ITS) region and the study of the morphology of the ring nematodes collected, the most abundant ring nematode species present in the stone fruit and grapevine production areas was concluded to be *C. xenoplax*. Another unknown species of ring nematode was recovered at a single site during the course of the study. *Criconemoides xenoplax* occurred in all the soil samples obtained during the survey, with nematode numbers ranging from 20 to > 2000 individuals per 250 ml of soil. *Criconemoides xenoplax* infestation in South Africa shows similar trends, as have been observed globally, demonstrating its importance as an economically significant pest. It is, therefore, essential to develop alternative management options to minimise the amount of damage incurred, and to manage *C. xenoplax* in the areas concerned, to sustain production.

Control of the ring nematode is difficult and is highly dependent on the use of chemicals. Alternative, more sustainable and eco-friendly management options, such as the use of resistant rootstocks in the grapevine and stone fruit industries, have become common practice. Six commercially available grapevine rootstocks were assessed for their susceptibility to *C. xenoplax* in a glasshouse pot trial. Additional data, from routine grapevine soil samples analysed by Nemlab, were used in conjunction

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with the glasshouse trial data. Grapevine rootstock susceptibility was evaluated by taking into account the reproductive factor (RF), and the nematode numbers recovered in the soil of the different rootstocks. The results of the glasshouse trial indicated a significant difference in grapevine rootstock susceptibility to *C. xenoplax*. Ring nematode numbers were recorded to be highest on the rootstock 110 Richter for both trials conducted in the glasshouse, whereas rootstocks 1103 Paulsen and 140 Ruggeri performance remained similar in both trials. Contrasting results were recorded between the 99 Richter and Ramsey rootstocks in the two trials, with Ramsey showing higher resistance than 99 Richter in the first trial, yet a lower resistance than 99 Richter in the second trial. The data analysed from Nemlab samples showed that the *C. xenoplax* numbers were generally higher from the field samples evaluated during the study. Further studies are required to further examine rootstock resistance to *C. xenoplax*. To do the above, however, a reliable source of *C. xenoplax* cultures needs to be available for future studies.

In addition to its preferred hosts, being grapevine and stone fruit, *C. xenoplax* has also been recorded on a variety of other plant species. Five annuals, specifically tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), mint (*Mentha*), carnation (*Dianthus caryophyllus*), white clover (*Trifolium repens*), and sweetcorn (*Zea mays* var. *saccharata*), were investigated in a glasshouse trial. The investigation was conducted to determine whether a monoxenic *C. xenoplax* population could be cultured *en masse* on alternative hosts, thus providing a more successful and rapid method of culturing the nematodes for future use. *Criconemoides xenoplax* was used to inoculate the host plants selected six weeks after replanting the seedlings, where after the plants were left for a duration of seven weeks to allow for nematode reproduction.

None of the annual hosts tested during the trial were considered a suitable host for the nematode, as no increases in the population were observed for the duration of the trial. As the RF values calculated were all below 1, using these annuals as an alternative option to culture *C. xenoplax* is not a viable option for future experiments. The use of grapevine and stone fruit plants should, as a result, remain the primary hosts for the sustained ring nematode populations in culture, with grapevine being the preferred host.

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To conclude, *C. xenoplax* is a significant nematode that requires more research in South Africa. Doing so would enhance the understanding and amount of knowledge pertaining to the biology of such an economically important pest, as well as promoting the understanding of future host damage and plant resistance. The above mentioned will be critical for the employment of efficient control methods to manage nematode populations in the future.

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OPSOMMING

Die ringaalwurm, *Criconemoides xenoplax* (Raski, 1952), Loof & De Grisse, word beskryf as 'n migrerende ektoparasitiese nematode wat hoofsaaklik op plantwortels van houtagtige meerjarige plante voed. Die ringaalwurm word wêreldwyd beskou as 'n beduidende plaag in steenvrugteboorde en wingerde, en dit het 'n algemene probleem in Suid-Afrikaanse produksiegebiede geword. Om die verspreiding van die ringaalwurm te bepaal, is 'n opname in wingerd- en steenvrugte produksiegebiede in beide die Wes- en Noord-Kaap provinsies uitgevoer. Grondmonsters is verkry deur lukraak geselekteerde plase te selekteer. Addisionele data wat ontvang was van die diagnostiese laboratorium, Nemlab, was ook ondersoek in verband met ringaalwurm voorkoms vanuit steenvrugte en wingerd gebiede. Ringaalwurms wat tydens die studie versamel is, is morfologies en molekulêr gekarakteriseer.

Na die DNS karakterisering van die ITS-streek asook die morfologiese studie, is dit bevind dat *C. xenoplax* die volopste ringaalwurm spesie is wat voorkom in die steenvrugte- en wingerdproduksiegebiede. Gedurende die studie is 'n onbekende ring-aalwurmspesie in een monster gevind. *Criconemoides xenoplax* het in 100% van die grondmonsters voorgekom wat tydens die opname versamel is, met aalwurm getalle wat gewissel het tussen 20 tot > 2000 individue per 250 ml grond. Met betrekking tot *C. xenoplax* besmetting, toon Suid-Afrika soortgelyke patrone as wat wêreldwyd gevind is en bewys dus die erns van die nematode as ’n ekonomies beduidende plaag. Om produksie te volhou, is dit noodsaaklik om alternatiewe bestuurs opsies te ontwikkel, wat ringaalwurm skade verminder deur die pes in die betrokke gebiede effektief te bestuur.

Die beheer van die ringaalwurm is 'n uitdagende taak, wat tans afhanklik is van die gebruik van chemikalieë. Alternatiewe bestuurs opsies, soos die gebruik van weerstandbiedende onderstokke in die wingerd- en steenvrugbedrywe, word meer geskik beskou weens hulle meer volhoubare en omgewingsvriendelike eienskappe. Die vatbaarheid van ses kommersiële beskikbare wingerd onderstokke vir *C. xenoplax* was geassesseer, in 'n glashuis pot proef. Bykomende data, wat verkry is tydens Nemlab se roetine analise van wingerd grondmonsters, is saam met die glashuisproef inligting gebruik. Onderstok vatbaarheid is geëvalueer deur die vermeerderings faktor (RF), en die aalwurm getalle wat in die grond van die verskillende onderstokke gevind was, in ag te neem. Die

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resultate van die glashuisproef het 'n beduidende verskil in onderstok vatbaarheid vir *C. xenoplax* getoon. In albei glashuis proewe, was die hoogste ringaalwurm getalle gevind op die 110 Richter onderstok, terwyl die 1103 Paulsen en 140 Ruggeri onderstokke soortgelyk presteer het in beide proewe. Die 99 Richter en Ramsey onderstokke het egter kontrasterende uitslae getoon tussen die twee eksperimente, met Ramsey wat meer weerstand gebied het teen die ringaalwurm in die eerste eksperiment, maar wel laer weerstand gebied het teenoor 99 Richter in die tweede eksperiment. Die data wat ontleed is vanuit die Nemlab monsters, het getoon dat die *C. xenoplax* getalle oor die algemeen hoër was as dié wat tydens die veldmonsterstudie geëvalueer is. Verdere studies word benodig om die onderstamweerstand teen *C. xenoplax* te bepaal. Om die bogenoemde te kan uitvoer, moet 'n betroubare bron van *C. xenoplax* kulture beskikbaar wees vir toekomstige studies.

Benewens sy voorkeurgashere wat wingerd en steenvrugte is, is gevind dat *C. xenoplax* ook op 'n verskeidenheid van ander plantspesies voorkom. Vyf eenjarige gewasse, spesifiek tamatie (*Lycopersicon esculentum*), blaarslaai (*Lactuca sativa*), kruisement (Mentha), angelier (*Dianthus caryophyllus*), wit klawer (*Trifolium repens*) en suikermielies (*Zea mays* var. Saccharata), is in 'n glashuis ondersoek. Die ondersoek is uitgevoer om vas te stel of 'n monokultuur *C. xenoplax* bevolking op alternatiewe gashere gekweek kan word, en sodoende 'n meer suksesvolle en vinnige metode te voorsien om die nematodes te kweek vir toekomstige gebruik. *Criconemoides xenoplax* is gebruik om gasheer plante, wat ses weke na die herplanting van die saailinge gekies is, te inokuleer, waarna die plante vir sewe weke in ’n glashuis gelaat is om aalwurm voorplanting te bevorder.

Geen van die jaarlikse gashere wat tydens die proef getoets was, word beskou as 'n geskikte gasheer vir die ringaalwurm nie, aangesien geen toename in die bevolking vir die duur van die eksperiment waargeneem is nie. Aangesien die berekende RF waardes almal onder 1 was, bewys dit dat hierdie jaarlikse plante nie as 'n alternatiewe opsie vir die kultuur van *C. xenoplax* gebruik kan word vir toekomstige eksperimente nie. Die gebruik van wingerd- en steenvrugplante, moet gevolglik die primêre gashere bly vir die aanteling van ringaalwurms, met wingerd wat beskou word as die voorkeur gasheer.

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Ten laaste, *C. xenoplax* is 'n problematiese plantparasitiese nematode en vereis dat meer navorsing op die pes gedoen moet word in Suid-Afrika. Verdere navorsing sal die insig en kennis, aangaande die biologie van die ekonomiese belangrike plaag verbeter, asook die kennis van toekomstige gasheerskade en plantweerstand bevorder. Om aalwurmbevolkings in die toekoms te bestuur, is die bogenoemde krities om doeltreffende beheermaatreëls te handhaaf.

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CHAPTER 1

A REVIEW OF THE RING NEMATODE, *CRICONEMOIDES XENOPLAX*, WITH SPECIAL REFERENCE TO GRAPEVINE AND STONE FRUIT

ABSTRACT

The ring nematode, *Criconemoides xenoplax,* is a migratory ectoparasitic nematode that feeds exclusively on the roots of plants, mainly woody perennials. The nematode is regarded as a significant pest in vineyards and stone fruit orchards worldwide. *Criconemoides xenoplax* has become increasingly problematic in South Africa, as it is the most common plant-parasitic nematode species found in grapevine and stone fruit. However, control of the ring nematode has proven to be a challenging task, due to its long life cycle and depth at which it occurs in the soil. Control relies heavily on the use of nematicides, while alternative control is focused on the use of resistant rootstocks in the grapevine and stone fruit industries. Different alternative methods need to be investigated to control ring nematodes. Hence, information on the biology, host damage, economic importance and management options of *C. xenoplax* is crucial to the implementation of efficient future control methods to decrease and manage nematode populations.

Key words: *Criconemoides xenoplax*, rootstock, review, stone fruit, grapevine.

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INTRODUCTION

Criconemoides xenoplax (Raski, 1952) Loof & De Grisse, 1967, known as a ring nematode, is a migratory, ectoparasitic, plant-parasitic nematode that completes its life cycle in the soil, on the roots of plants (Seshadri, 1964; Core, 2001; Cordero *et al*., 2012). Although the nematode has a wide host range, it has only been reported as a pathogen on a few documented hosts. *Criconemoides xenoplax* is a known parasite of grapevine (Raski & Radewald, 1958) and stone fruit (*Prunus* spp.) (Nyczepir, 1991), including apricot, peach (Lownsbery *et al*., 1973; Nyczepir *et al*., 1983), cherry (Thorne, 1955), plum (Goodey & Franklin, 1956), cranberry (Bird *et al*., 1964) and spruce (Epstein & Bravdo, 1974; Mojtahedi & Lownsbery, 1976).

Criconemoides xenoplax and several other nematode species in the same suborder have been recorded as causing significant damage to agricultural production in a number of countries (Subbotin *et al*., 2005). Numerous studies have identified *C. xenoplax* as a key factor that is responsible for the occurrence of a disease complex referred to as Peach Tree Short Life (PTSL) in stone fruit and for the reduced growth of vineyards worldwide. Nyczepir *et al*. (1983) were the first researchers to prove the association of *C. xenoplax* with the PTSL syndrome. However, Raski & Radewald (1958) were the first researchers to demonstrate accurately the true parasitic nature of the ring nematode.

In South Africa, exceptionally high numbers of *C. xenoplax* have been reported from three different diagnostic laboratories (Nemlab, ARC diagnostic laboratory and Nemconsult). A ten-year stone fruit rootstock evaluation study for the screening of *C. xenoplax* has been conducted in 2013, resulting in five stone fruit rootstocks being selected as tolerant to ring nematode, with no resistant rootstocks having yet been found (Booi & Malan, 2013). In the case of grapevine in South African the tolerance status of rootstocks is relatively unknown.

Currently, the stone fruit industry is largely dependent on the use of nematicides to control *C. xenoplax*. However, due to the adverse effects of some nematicides on the environment and

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the increased restrictions regarding their use, alternatives are currently being investigated to minimise the use of chemicals to control plant-parasitic nematodes. The use of alternative management approaches relies on the sound knowledge of nematode biology and taxonomy (Perry & Moens, 2006). In the case of *C. xenoplax,* much uncertainty and controversy has existed regarding its systematics and taxonomic status. Although numerous studies regarding the morphology of *Criconemoides* are available, there is still insufficient data on *C. xenoplax* taxonomy. Identifying the nematodes correctly is essential to ensure their proper management, due to their association with the damage of economically important crops (Cordero *et al.*, 2012). Developing a catalogus of the morphological and molecular status of different *C. xenoplax* populations from different geographical regions should therefore, aid in the understanding of the host- parasite relationship (Subbotin *et al*., 2005).

The main aim of this chapter is to provide a current compilation of information regarding the ring nematode complex. Information on different aspects of *C. xenoplax* is consolidated in this review, including their taxonomy, biology, control options and association with stone fruit and grapevine.

IDENTIFICATION

Despite the intensive study of the morphological taxonomy of the criconematids, such varying interpretations of the characters exist that taxonomists have not yet been able to reach consensus with regard to the validity of genera and proposed species. More than 750 ecto- and semi-endo plant-parasitic nematode species are included in the suborder Criconematina (Siddiqi, 1980). Currently, molecular methods combined with traditional morphological methods, are providing an accurate and reliable method of identification of species within a specific groups of nematodes (Subbotin *et al*. 2005).

The systematics of the Criconematinae is complicated by the numerous taxonomic and diagnostic problems existing at both species and genus level. De Ley *et al*. (2005) analysed individual nematodes from two different areas in California, using the Ribosomal Transcribed

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Spacer (ITS) as well as the D2/D3 expansion domains of the nuclear 28S rDNA subunit The results obtained indicated the existence of two variants, differing by eight base pair substitutions in the ITS region. Genetic differences were supported by means of morphological differences detected in the length of the stylet.

In 2012, Cordero *et al*. studied the taxonomic and molecular identification of *Mesocriconema* and *Criconemoides* species, also using the ITS1-rDNA region for interspecies comparison. However, Cordero *et al*. (2012) showed that the ITS-rRNA sequences were too variable to allow for the successful study of the phylogenetics of Criconematoidea, despite being useful for the identification and characterisation of species within families (Table 1.1). The researchers suggested that such markers as mitochondrial DNA (COI and COII) should also be incorporated to give a better understanding of the phylogenetic relationships involved.

Table 1.1. List of primers used for different regions in the molecular identification of *Criconemoides xenoplax*.

Region	Primers	Reference
ITS1-rDNA	rDNA2: (18S)-TTGATTACGTCCCTGCCCTTT 3'(forward) (Vrain *et al*., 1992) rDNA1.58s: 5'-GCCACCTAGTGAGCCGAGCA- 3' (reverse) (Cherry *et al*., 1997).	Cordero *et al*., 2012; 2013
18S rDNA	18S1.2a: 5'-CGATCAGATACCGCCCTAG-3' (forward) 18Sr2b:5'-TACAAAGGGCAGGGACGTAAT-3' (reverse)	Powers *et al*., 2010
ITS1-rDNA	rDNA2:5'-TTGATTACGTCCCTGCCCTTT-3'(forward) rDNA1.58Sa: 5'-ACGAGCCGAGTGATCCACC-3' (reverse)	Powers *et al*., 2010
Mitochondrial cytochrome b - cytb)	CytB1F: 5'-KDAATTTTGGKAGWWTWYTRGG-3' (forward) CytB1R: 5'-AGCACGYAAAATWSCRTAAGC-3' (reverse) (Nieberding *et al*., 2005).	Powers *et al*., 2010
28S rRNA	D2A (5-ACAAGTACCGTGAGGGAAAGTTG-3 (forward) D3B (5'-TCGGAAGGAACCAGCTACTA-3' (reverse)	Subbotin *et al*., 2005; 2006

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DISTRIBUTION

The ring nematode, *C. xenoplax* was initially described from samples obtained near Fresno, California from the roots of Thompson seedless grapes by Raski (1952). Since its discovery, *C. xenoplax* has been reported from regions of North and South America, Australia, Africa, Europe (Weischer, 1960), India (De Grisse, 1968), and Japan (Core, 2001). The nematode is the most widely distributed species amongst the genus *Criconemoides* (Seshadri, 1964) and is regarded to be a significant pest in stone fruit orchards (Nyczepir *et al*., 1983) and vineyards worldwide (Pinochet & Cisneros, 1986).

Ring nematode species are associated with a diverse range of plants, of which woody perennials are the most favourable host. Their distribution has been reported to be worldwide, so that they are present in roughly every area where nematode research has been conducted (Seshadri, 1964; Raski & Golden, 1965). The extensive distribution of *C. xenoplax* and *C. curvatum* has been recorded from many regions of Africa, Asia, Europe, North and South America (Weischer, 1960; Keetch & Heyns, 1982; Pieterse & Meyer, 1987; Pinkerton *et al*., 1999; Aballay *et al*., 2009).

In South Africa, ring nematodes have been recorded as being present in vineyards, stone fruit orchards, plantations and forests, as well as in gardens and virgin fields. Heyns (1970), however, was the first to identify *C. xenoplax*, together with a number of other Criconematinae species. *Criconemoides xenoplax* is described as being the most common ring nematode species occurring in vineyards in the Western Cape area (Keetch & Heyns, 1982). Marais and Swart (2001; 2002) went on to record its presence in the Northern Cape and Modimolle area, Limpopo province. Its abundance in the Northern Cape was 67% and 75% in vineyards and orchards respectively, and in the Modimolle area, it was also recorded to be associated with grapevine and orchards, where it was found to have an incidence level of 32%.

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BIOLOGY

Once a ring nematode comes into contact with the roots of the host, it locates the root epidermis by means of probing until a suitable area for feeding is found, resulting in the penetration of the surface of the host root by the stylet. Feeding sites are established by the nematode at a specific cell, which is referred to as a 'food cell'. The cells concerned are usually located in either the first or second cortical cell layers, with them showing characteristic modifications to the ultrastructure that provides the nematodes with the needed nutrients. The stylet tip is usually inserted into the food cell through the wall, avoiding piercing of the plasma membrane. Nutrients are withdrawn directly from the cytosol in the food cell, as a result of the close association of the wall of the stylet with the membrane, with the nutrients flowing through an opening formed in the plasma membrane (Hussey *et al*., 1992).

As the entire life cycle of *C. xenoplax* occurs outside the host root, the ectoparasite is referred to as being migratory (Core, 2001). In the study carried out by Westcott & Hussey (1992), *C. xenoplax* were found to feed continuously at a specific root cortical cell for a period of from 1-8 days, without causing damage to the root cell. They illustrated that no necrosis occurred along the root as a result of the feeding of a single nematode. Parasitised roots were, thus not identifiable from the presence of necrotic tissue. The observed feeding behaviour of *C. xenoplax* is considered to be more highly evolved than is the feeding behaviour of many other nematodes that feed on a number of cells within a short period of time (Westcott & Hussey, 1992). The life cycle, under laboratory conditions, is estimated to take 24 to 30 days, of which almost half of the time is spent in the egg stage. The process of oviposition is completed over a period of two to three days. Eggs are usually deposited on the surface of the roots, or in close proximity to the host roots (Thomas, 1959; Seshadri, 1964; Core, 2001).

Nyczepir *et al*. (1988) discovered that *C. xenoplax* is able to produce the enzymes β-cyanoalanine and β-glucosidase, of which the former is responsible for the detoxification of the release of cyanide from prunase, with the latter being released to play a role in prunasin

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metabolism. The release of β- cyanoalanine thus enables the establishment of *C. xenoplax* populations on peach trees.

EFFECTS OF SOIL ENVIRONMENT

As nematodes are aquatic organisms, their activities are dependent on water. Populations are limited to inhabiting soil spaces, with their movement, which is limited by their body size, leaving the soil undisturbed. Nematodes can only move through pores that are wide enough to accommodate the width of their bodies (Wallace, 1963). Therefore, soil factors affecting nematode distribution include temperature, soil moisture, pore size, and aeration (Wallace, 1963).

TEMPERATURE AND SOIL MOISTURE

The optimum conditions required for *C. xenoplax* reproduction in orchards and vineyards include both high temperatures and high rainfall patterns, as recorded by Pinochet and Cisneros (1986) in three different Spanish vineyards. The *C. xenoplax* population was seen to increase during periods of high rainfall and high temperatures, suggesting that both climatic factors are key to influencing *C. xenoplax* numbers (Pinochet & Cisneros, 1986). Thus, the combined effect of high temperature and high rainfall results in the optimum conditions being present that are necessary for nematode reproduction. A decrease in *C. xenoplax* populations was observed to occur during peak hot months when the precipitation was low. A similar result was obtained when high precipitation occurred in combination with low temperatures. Nematodes, however, do manage to persist and to remain active, albeit it in relatively low numbers, under unfavourable conditions (Pinochet & Cisneros, 1986). Nesmith *et al*. (1981) also recorded the presence of high numbers of *C. xenoplax* during both summer and midwinter, when soil moisture and temperatures were high. The conclusion can, therefore, be drawn that soil moisture is the main driver for *C. xenoplax* reproduction (Nesmith *et al*., 1981).

Soil temperature plays an important role in the relationship between parasites and their host plants. Temperature affects the rate of physiological processes, which includes growth and

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host plant response to infection, as well as the population increase of nematodes. Host and parasite interaction might also be temperature-sensitive, as it is regulated by the expression of certain genes (Griffin, 1969; Jatala & Russell, 1972; Thies & Fery, 1998; Ferris *et al*., 2013). Most phytoparasitic nematodes are believed to be inactive below 15°C and above 30°C. Thus, optimum temperatures tend to lie between 15°C and 30°C. For instance, Lownsbery (1961) recorded an optimum temperature of 26°C for *C. xenoplax* reproduction.

Despite being aquatic organisms, nematode populations are negatively impacted by extreme moisture settings (Wallace, 1963). *Criconemoides xenoplax* populations are assumed to be highly dependent on rainfall, with soil moisture being recorded as being an important factor affecting the size of their populations by a number of authors (Lawrence & Zehr, 1978; Nesmith *et al*., 1981). Lawrence & Zehr (1978) note that soil moisture has been observed to have a significant effect on *C. xenoplax* numbers, although soil moisture levels vary in different soil types. Very high numbers of *C. xenoplax* was recorded when the nematodes were extracted from soil samples during wet periods, with soil moisture levels ranging between 16-24%, compared to the numbers that were extracted during dry periods, when the soil moisture levels were low.

SOIL TYPE

Soil types might have an influence on nematode populations, as they tend to have different moisture-retaining characteristics (Wallace, 1963). Malossini *et al*. (2011) carried out a study to determine the distribution of *C. xenoplax* in the soil of vineyards in two localities in Italy. The researchers found that the vertical distribution of *C. xenoplax* was highly dependent on plant root systems and, consequently, on the soil texture. In sandy soils, roots tend to grow to deeper levels underground, as a result higher nematode densities were found at deeper levels compared to populations found in compact soils. Nematode densities, as a result of the soil texture were found to be higher in the upper levels of compact soils when compared to densities that were found at lower levels (Malossini *et al*., 2011).

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HOST DAMAGE AND ECONOMIC IMPORTANCE

GRAPEVINE

Grapevine (*Vitis vinifera*), which is the most widespread fruit crop worldwide, originated in Asia Minor and the Caucasus region. It is currently being found in areas with Mediterranean and temperate climates throughout the world. Grapevine production has moved within and between continents concurrently with the migration of humans, with grapevines currently being produced in a number of different regions. Such a distribution includes Europe, South and North America, Southern Africa, the Mediterranean basin and the subtropical areas of Australia (Téliz *et al*., 2007; Van Zyl & Walker, 2012). Viticulture within South Africa is practice in ten regions, however, 90% of the production is situated in the Western Cape province. In South Africa a total area of 95 775 ha (SAWIS, 2017) and 18 674 ha (SATI, 2017) is planted for wine grapes and table grapes respectively, making up a total value of R15.4 billion for export. The increased production of grapevine has resulted in the amplified spread and frequency of related diseases and pests observed worldwide (Van Zyl & Walker, 2012; Ferris *et al*., 2013).

Criconemoides xenoplax, which has a high frequency and a broad-based distribution throughout grape production areas, is regarded as an economically significant pest in vineyards worldwide, due to grapes being a highly favourable host (Keetch & Heyns, 1982; Pinkerton *et al*., 2005). The ring nematode concerned has been found in 75% of Germany's vineyards (Weischer, 1960) and in 98% of Switzerland's vineyards (Pinkerton *et al*., 1999), with it also being found abundantly in Australian (Walker 1995), Italian (Malossini *et al*., 2011), Spanish (Pinochet & Cisneros, 1986), South Africa (Smith, 1977) and French (Scotto La Massese et *al*., 1973) vineyards. *Criconemoides xenoplax* is recorded as widespread in vineyards located throughout the Western Cape region (Smith, 1977) with more recent surveys reporting higher densities of *C. xenoplax* populations, due to the availability of more advanced extraction methods. A 90% occurrence of the nematode has been recorded in

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vineyards soils within the Western Cape, with *C. xenoplax* being described as the key pest responsible for the damage observed on grapevine (Storey *et al.,* 2017).

Knowledge of the impact that *C. xenoplax* can have on the productivity and growth of vines is still lacking and where available, is poorly understood (Pinkerton *et al*., 2005). The majority of studies that have so far been conducted have resulted in varying conclusions, including those that have been undertaken by Santo & Bolander (1976), Téliz *et al*. (2007), McKenry & Anwar (2006) and Schreiner *et al*. (2012). Klingler (1975) concluded that the presence of *C. xenoplax* results in reduced grapevine vigour. Storey *et al*., (2017) reported more root sprouting from the sides on plants parasitized by ring nematode, the roots are described as discoloured, short and dead (‘witches broom’). However, other researchers such as Raski & Radewald (1958) and Pinkerton *et al*. (1999) found no correlation between nematode population and grapevine vigour. McKenry (1992) estimated that *C. xenoplax* populations of over 500 nematodes per kg (125 ring nematodes / 250 ml) soil tend to reduce grape yields in California by 10 to 15%.

The relationship between the population density of nematodes and damage to plants is, in many cases unclear. The poor health of vineyards might be due to individual factors, or due to a combination of factors such as vineyard age, cultivar and other stressors. The latter could include the existence of poor soils, due to the presence of disease and other pests, water stress or type of rootstock used. Feeding by *C. xenoplax* caused destruction of root systems, leading to rapid darkening and underdeveloped root systems, with a decreased presence of feeder roots (Santo & Bolander, 1976; Pinkerton *et al*., 1999). Klingler (1975) went on to report that *C. xenoplax* destruction was greatest on the relatively young roots. Nematode attacks tend to occur in large numbers and often locally on roots, causing root tissue destruction, with the complete breakdown of infected roots occurring over a period of days. Despite nematode activity not resulting in the thickening and deformation of grapevine roots, comparatively few fine roots were observed (Klingler, 1975).

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Santo and Bolander (1976) observed the stunting of potted concord plants infected with high numbers of *C. xenoplax*. High numbers of ring nematode negatively affected the fresh weight of both the roots and the tops of grapevine, which tends to decrease in correlation with an increase in nematode population numbers. However, the inoculation of relatively low nematode numbers only inhibited top growth, without it seeming to have an effect on root growth. Similarly, Schreiner *et al*. (2012) noted a reduction in the growth of fine roots and an increase in arbuscular mycorrhizal fungi (AMF) colonisation on susceptible and self-rooted vines.

Conversely, Nigh (1965) reported that *C. xenoplax* neither reduced root weight, nor caused noticeable symptoms on the roots of Thomson seedless grapes in glasshouse experiments. Pinkerton *et al*. (1999) suggest that damage by nematodes is only evident when vineyards are exposed to other stresses, including such factors as over cropping, poor soil conditions and water stress.

STONE FRUIT (PEACH, PLUM AND APRICOT)

A total of 90% of South African stone fruit production occurs within the Western Cape province, with stone fruit production being an important commodity in the Western Cape region for over a century. However, due to the limited amount of virgin land, replanting of stone fruit crops have resulted in the increased build up of plant-parasitic nematodes over the years. One such nematode, *C. xenoplax,* was documented as the key nematode pest on apicot in South Africa, based on the results of a survey conducted by Meyer in 1976. The nematode was present in 40% of the 89 orchards sampled, with populations exceeded 500 individuals per 250 cm^3 of soil in 50% of the samples analysed (Hugo & Storey, 2017). Meyer (1973) regarded *C. xenoplax* as the most important nematode pest in orchards as a result of long term investigative experience. Between the years of 1882 and 2010, several other researchers regarded *C. xenoplax* amongst others, as a significant plant-parasitic pest on the roots of peach and nectarines in South Africa. As *C. xenoplax* was the only nematode recovered in

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the majority of the soil samples collected and the numbers usually exceeded 1 000 individuals per 250 cm³ soil (Hugo & Storey, 2017).

In the south-eastern United States, the productive life span of peach trees does not exceed 6 to 10 years on some sites, due to premature tree mortality. Therefore, the full production potential of many orchards is never reached, as a total of almost 95% of the trees is expected to die in a heavily infested PTSL site (Brittain & Miller, 1978; Wehunt *et al*., 1980; Nesmith *et al*., 1981). The external symptoms of PTSL are similar to those of any plant that is deprived of an adequate root system (Taylor *et al*., 1970).

PTSL is thought to occur as a result of a number of predisposing interacting factors, both physical and biological, including the presence of high ring nematode populations. Nesmith & Dowler (1975), Zehr *et al*. (1976), and Nyczepir *et al*. (1983) were the first to prove the important role that *C. xenoplax* plays in PTSL. Since then, *C. xenoplax* has been identified as the major predisposing factor that is associated with PTSL by a number of other researchers. PTSL is a condition that is characterised by the rapid death of seemingly healthy peach trees seen after, before or during bloom. The death and rapid collapse of the above-ground parts of trees usually occurs between the third and fifth years (Wehunt *et al*., 1980; Ritchie & Clayton, 1981).

High populations of *C. xenoplax* predispose the tree to PTSL, in combination with certain environmental conditions, such as the variety of rootstock, cold temperatures, the interaction of soilborne microorganisms interacting with a high nematode population and poor management practices. Such practices include replanting in old peach production areas, early pruning, poor drainage, salinity and nutrient deficiency, with the factors concerned all serving to weaken and predispose trees to bacterial canker and cold injury (Ritchie & Clayton, 1981). A high number of infestations with *C. xenoplax* causes the trees involved to become highly susceptible to cold damage and to infection with bacterial cankers *Pseudomonas syringae* pv. *syringae*, which commonly leads to the direct death of the trees concerned (Nesmith *et al*.,

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1981; Ferraz & Brown, 2002). As resistance to parasitism and cold injury were observed in healthy trees, the different factors that predispose trees are important in disease development (Nesmith & Dowler, 1975; Zehr *et al.*, 1976).

The combined interaction of the different environmental factors and management practices with a high nematode population tends to lead to root destruction that is primarily brought on by the parasitism of nematodes. Damage to the root systems of plants later leads to an observed reduction in the yield and growth of plants (Mai & Abawi, 1981; Ferraz & Brown, 2002). The extent of damage that occurs to host plants as a result of nematode infestation is dependent on the reproduction rate, the population density and the tolerance of the host plants (Seinhorst, 1970).

Host plant damage caused by plant-parasitic nematodes depend on three factors, namely the host tolerance, the rate of reproduction and the population density (Nyczepir *et al*., 1987). Nyczepir *et al*. (1987) conducted a study to determine the effects of the initial *C. xenoplax* population density on amino acids, reducing sugars and peach seedling survival over time. The population increase of *C. xenoplax* was subsequently recorded as occurring at a relatively high rate in the soil that was inoculated with the lowest initial population. The number of free amino acids found in the root was recorded to increase with an increase in *C. xenoplax* numbers. The suppression of such plant characteristics as height, weight and root volume was also observed, together with a decrease in the number of reducing sugars in the root tissue. However, the behaviour observed in the plants was recorded as occurring at a relatively late stage and when pruning occurred (Nyczepir *et al*., 1987). The changes observed in the peach seedlings were attributed to the initial *C. xenoplax* populations and to their reproduction rates (Nyczepir *et al*., 1987).

The damage threshold for *C. xenoplax* in peach orchards in sandy soils in the south- eastern USA is 50 to 100 nematodes / 100 cm^3 soil (Nyczepir *et al.,*1983). Pre-plant fumigation using

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a broad-spectrum soil fumigant on a site that was infested with *C. xenoplax* and prone to PTSL syndrome increased total yields over a three-year period by more than 10 000 kg/ha.

The inoculation of a plum cultivar, Myrobalan 19C with *C. xenoplax* resulted in reduced potassium and phosphorus levels in leaves, with an overall reduction in the fresh weight of leaves. Roots infested with nematodes are generally dark in colour, as well as lacking in feeder roots. Other symptoms of infestation recorded, include the disintegration of the stele with a darkening of the sites where feeding occurred, owing to the oxidisation of the phenolic compounds present (Mojtahedi & Lownsbery, 1974). Plant susceptibility to bacterial canker and cold injury is believed to increase with heavy parasitism by nematodes, which interrupts the partitioning of carbohydrates and which affects the phenol oxidase processes.

In the Western Cape, rootstocks of stone fruit used for resistance to root-knot nematode were found to be highly susceptible to the depredations of ring nematode, especially in the cases of the rootstock Marianna for plum and Nemaguard for peach (P. Stassen per. comm.). High numbers of ring nematode, along with adverse environmental conditions, tended to predispose the trees to infestation with such wood-rotting fungi as *Leucostoma* and *Botryospherea.*

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Table 1.2. Reported resistance (R), susceptibility (S) and tolerance (T) of rootstock cultivars to *Criconemoides xenoplax* surmised and categorised by means of extrapolation from information contained in the literature.

Common name/genus/ species	Rootstock/ cultivar/cross	R	T	S	Country	Reference
Grapevine/ *Vitis vinifera*	101-14	X	-	-	USA	Beckman *et al*., 1993 Okie *et al.*, 1994; Westcott & Zehr, 1991; Schreiner *et al.*, 2012.
	Columbard	-	-	X	USA	Ferris *et al*., 2013
	Harmony	-	-	X	USA	Ferris *et al*., 2013; McKenry *et al.*, 2001
	Jacques	-	-	X	SA	Pieterse & Meyer, 1987b
	M. rotundifolia (Dowart)	X	-	-	USA	Ferris *et al*., 2013
	M. rotundifolia (Trayshed)	X	-	-	USA	Ferris *et al*., 2013
	Ramsey	-	-	X	USA	Beckman *et al*., 1993; Okie *et al.*, 1994; Westcott & Zehr, 1991
	Richter 110	X	-	X	SA, USA	Pieterse & Meyer, 1987b; Beckman *et al*., 1993 Okie *et al.*, 1994; Westcott & Zehr, 1991
	Richter 99	-	-	X	SA, USA	Pieterse & Meyer, 1987b; Beckman *et al*., 1993; Okie *et al.*, 1994; Westcott & Zehr, 1991; Mckenry *et al.*, 2001
	Ruggeri	-	-	X	SA	Pieterse & Meyer, 1987b
	Salt Greek	-	-	X	SA	Pieterse & Meyer, 1987b
	St George	-	-	X	USA	Ferris *et al*., 2013
	UCD-GRN1 (*V. rupestris* x *M. rotundifolia*)	X	-	-	USA	Ferris *et al*., 2013
	Concord	-	-	X	USA	Santo & Bolander, 1976
	Thomson seedless	-	-	X	USA	McKenry *et al.*, 2001
	Ramsey	-	-	X	USA	McKenry *et al.*, 2001
	Freedom	-	-	X	USA	McKenry *et al.*, 2001
	Flame seedless	-	-	X	USA	McKenry *et al.*, 2001

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Common name/genus/ species	Rootstock/ cultivar/cross	R	T	S	Country	Reference
Peach	Guardian	-	X	-	USA	Beckman *et al*., 1993; Okie *et al.*, 1994; Westcott & Zehr, 1991)
	Nemaguard	-	-	X	USA	Zehr *et al.*, 1976; Okie *et al.*, 1994
	Kakamas	-	-	X	SA	Hugo, 2017
	Viking	-	X	-	SA	Hugo, 2017
	GF 677	-	-	X	SA	Hugo, 2017
	FloraGuard™	-	-	X	SA	Hugo, 2017
	Lovelle	-	X	-	USA	Zehr *et al.*, 1976; Okie *et al.*, 1994
	Elberta	-	-	X	USA	Zehr *et al.*, 1976
Apricot	Royal	-	X	-	SA	Hugo, 2017
	Salome	-	X	-	SA	Hugo, 2017
Plum	Marianna	-	X	X	SA, USA	Beckman *et al*., 1993; Okie *et al.*, 1994; Westcott & Zehr, 1991; Mojtahedi & Lownsbery, 1974, Hugo, 2017
	Maridon	-	-	X	SA	Hugo, 2017
	Myrobalan	-	-	-	USA	Mojtahedi & Lownsbery, 1974
Cherry	Mazzard	-	-	X	USA	Melakeberhan *et al.*, 1994
	Mahaleb	-	-	X	USA	Melakeberhan *et al.*, 1994
	G1148-1	-	-	X	USA	Melakeberhan *et al.*, 1994
	G1148-8	-	-	X	USA	Melakeberhan *et al.*, 1994
Cranberry		-	-	X	USA	Bird & Jenkins, 1964
Spruce		-	-	X	USA	Epstein & Griffin, 1962
Mint		-	-	X	USA	Merrifield, 1991
Crimson clover	*Trifolium incarnatum* L. var. elatius Gibbelli & Belli 'Dixie'	-	-	X	USA	Westcott & Hussey, 1992
Dwarf English trefoil / *Lotus corniculatus* var. *arvensis*		-	-	-	USA	Zehr *et al.*, 1990
Big trefoil / *L. uliginosis*		-	-	X	USA	Zehr *et al.*, 1990

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Common name/genus/ species	**Rootstock/ cultivar/cross**	**R**	**T**	**S**	**Country**	**Reference**
Birdsfoot trefoil / *L. corniculatus*		-	-	X	USA	Zehr *et al.*, 1990
Narrowleaf birdsfoot / *L. tenuis*		-	-	X	USA	Zehr *et al.*, 1990
Ball clover		-	-	X	USA	Zehr *et al.*, 1990
Rose clover/Trifolium hirtum All		-	-	X	USA	Zehr *et al.*, 1990
Subterranean clover		-	-	X	USA	Zehr *et al.*, 1990
Striate lespedeza		-	-	X	USA	Zehr *et al.*, 1990
Partridge pea		-	-	X	USA	Zehr *et al.*, 1990
Carnation	*D. caryophyllus*	-	-	X	USA	Westcott & Hussey, 1992
Tomato	*Lycopersicion esculentum Mill. 'Rutgers'*	-	-	X	USA	Westcott & Hussey, 1992
Pecan	*Carya illinoinensis*	-	-	X	USA	Nyczepir & Wood, 2008
Walnut	*Juglans regia L. cv. Bleggiana*	-	-	X	Italy	Ciancio & Grasso, 1998

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NUTS

Pecan nuts, *Carya illinoinensis,* are cultivated in many countries including South Africa. *Criconemoides xenoplax* was found to be associated with pecan trees by Kleynhans (1986) in South Africa and by Nyczepir & Wood (2008) in Georgia. However, Nyczepir & Wood (2008) concluded that the amount of root damage that was done to pecan trees was greater in the case of trees affected by both *C. xenoplax* and *Meloidogyne partityla* Kleynhans, 1986 than that which was done to trees inoculated with *C. xenoplax*.

Walnut has also been recorded to be affected by *C. xenoplax*. Lownsbery *et al.* (1978) performed a trial using the Northern California black walnut, *Juglans hindsii Jeps*, and Persian walnut *J. regia L.* Plant growth was significantly reduced in the affected trees, compared to the amount of growth that occurred in nematode-free trees. Such reduction in growth was the result of the necrosis of the feeder roots, abrasions in the secondary phloem and longitudinal lesions occurring in the older roots. When Ciancio & Grasso (1998) also studied the effects of *C. xenoplax* on walnut, they found similar results to those of Lownsbery *et al.* (1978). The epidermis and cortex layers were damaged, with cell disruption due to *C. xenoplax* activity being found to have occurred both on and in the root systems concerned.

CONTROL AND MANAGEMENT OPTIONS

Site selection is an important step in managing nematode populations. Sites that favour the growth of stone fruits and that have no previous history of nematode and stone fruit problems, are preferred. However, if nematode-free locations do not exist, proper management practices should be applied. Management strategies include both pre- and post-plant chemical control, fallowing, crop rotation, the use of resistant rootstocks and biological control by means of entomopathogenic nematode and other soilborne microorganism use (Stirling, 1991).

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CHEMICAL CONTROL

The use of nematicides and fumigants has been the main control method employed in managing *C. xenoplax* numbers in both vineyards and orchards worldwide. The effectiveness of fumigation has been due to the volatility of the toxin and to its diffusion throughout spaces in the soil matrix (Mai & Abawi, 1981). However, due to the harmful effects recorded in terms of both human and environmental health, the amount of nematicide use has been reduced. The reduction has resulted in the increased loss of orchards to PTSL by almost three times the level expressed when the most efficient nematicide was used (Kluepfel *et al*., 2002; Perry & Moens, 2006).

Sharpe *et al*. (1988), on studying the effects of soil fumigation on peach tree establishment in replant sites concluded that the nematode population decreased with the application of methyl bromide, together with a decrease in the loss of the number of trees to PTSL and an increase in tree circumference and growth.

The implementation of pre-plant fumigation and post-plant nematicide application has been found to lead to a decrease in the number of plant-parasitic nematodes to undetectable levels (Nyczepir *et al*., 1983). The application of nematicides has also been found to increase the health and vigour of root systems and to decrease the extent of tree loss to cold hardiness and bacterial canker. However, the reduced loss of trees was observed by Zehr *et al*. (1976), due to the combination of a decreased number of nematodes, together with rootstock resistance. Although the application of nematicides of less resistant rootstocks did not inhibit tree loss to bacterial canker and cold injury, the extent of tree loss was nonetheless, lower than was that of tree loss without chemical application (Zehr *et al*., 1976).

For pre-plant fumigation and post-plant nematicide application to be effective requires both expenditure and long-term commitment, as such alternatives as rootstock resistance are currently being developed (Beckman *et al*., 1993).

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RESISTANT ROOTSTOCKS

The placing of emphasis on the need to develop host plant resistance has come about as a result of the restrictions placed on nematicide use and availability. Host plant resistance is seen by many as a solution to reducing the amount of destruction caused by nematodes. Such resistance is also due to the increased access to and the improved availability of plant germplasms containing resistant genes, following on the rapid advances made in technological development (Roberts, 1992). Although host plant resistance to nematodes has been developed in a number of crops, it is most effective against the more specialised species and genera of sedentary endoparasites, such as root-knot, cyst and citrus nematode.

As was previously observed by Zehr *et al*. (1976), the extent of tree loss to PTSL was greatly reduced with the use of a relatively resistant rootstock. Lovell rootstock, which is seen to be an important factor in managing PTSL is recommended for use in areas that are infested with nematodes. As Aballay *et al*. (2009) noted, there has, as yet, not been a rootstock that has been recorded as being fully resistant to *C. xenoplax*.

In South Africa, the stone fruit industry is highly dependent on a number of commercially imported rootstocks. However, such rootstocks in many cases are not adapted to survive the local climatic conditions and soil types (Booi & Malan, 2013). As a result, continual improvement is required in developing the resistance of crops to and their tolerance against, pests and diseases. The two main pests, *C. xenoplax* and *Meloidogyne javanica* cause significant economic loss in the fruit industry.

Rootstock tolerance or resistance in grapevine is seen as an attractive management option for use in protecting against *C. xenoplax* (Pinkerton *et al*., 2005). Rootstocks have been used in viticulture for over 150 years, to protect plants against soil pests. Over the years, research has focused on developing rootstocks that provide extensive and long-lasting resistance to grapevine pests (Ferris *et al*., 2013) and orchards (Zehr *et al*., 1976). Variations occur in the

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growth, longevity and survival of different rootstock varieties, as well as within families due to external factors such as site selection and genetic variation (Beckman *et al*., 1993).

Planting nematode resistant rootstocks has proven to be the most cost-effective means to maintain high grape productivity in infested soil (Winkler *et al*., 1974). However, the degree of resistance among rootstocks may vary between different ring nematode populations (Cain *et al*., 1984). McKenry (1992) recorded reduced yields of up to 25% if ring nematode populations exceed 500 per kg soil, or as a result of specific site conditions (Nicol *et al.*, 1999). Pinkerton *et al*. (2005) identified rootstocks with resistance to and/or tolerance of populations of *C. xenoplax* in the Pacific north-west.

Schreiner *et al*. (2012) conducted a four-year study to test and observe the response of self-rooted, resistant and susceptible rootstocks to ring nematode parasitism. Included in the study were six rootstocks, with two being categorised as very resistant (420A, 101-14), one with adequate known resistance (Richter 110) and three suitable rootstocks, including a self-rooted vine. The results showed that the self-rooted vines and susceptible vines were more adversely affected by ring nematode parasitism. The populations concerned had rapidly increased by the second year, remaining high during the course of the study. Rootstocks Richter 110 and 101-14, which were previously thought to be resistant, showed an increase in nematode populations towards the third year. However, 420A was the only rootstock that showed resistance throughout the four-year study period (Schreiner *et al*., 2012).

BIOLOGICAL CONTROL

Ectoparasitic plant-parasitic nematodes tend to spend their entire lifespan in the soil, which is described as being a unique and complex environment. Their activity is thus subjected to variations in both the physical elements of the soil, including moisture, temperature and aeration, as well as to changes in the composition of soil biota. Such living organisms include bacteria, fungi, algae, insects, other nematodes and a vast array of other soil organisms. Jaffee and Zehr (1983) recorded a decline in *C. xenoplax* numbers under favourable

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conditions suggesting predation or parasitism. Thus, the activities of soil borne organisms can be seen to play an important role in keeping nematode populations more or less constant. Such comparative constancy is achieved by way of predation and competition for resources both within and between the soil organisms present (Stirling, 1991).

Biological control makes use of microbial organisms to manage plant diseases and pests. Such management requires comprehensive knowledge of the target pest, including understanding their natural enemies' population dynamics and the interaction between the two organisms (Perry & Moens, 2006). The study of the potential of soilborne microbes as biological control agents has only recently been investigated in an effort to regulate plant-parasitic nematodes. Although, over the years biological control has focused more on endoparasitic nematodes, however the control of such nematodes by means of the use of root- colonising microorganisms has proved to be extremely difficult. Ring nematodes in contrast, are ectoparasitic with their entire life cycle being exposed to the environment of the rhizosphere. Thus, controlling ring nematodes with the use of rhizosphere-inhibiting microorganisms is attractive to many (Becker *et al*., 1988; Kluepfel *et al*., 2002).

BACTERIA

Much of the life cycle of ectoparasitic plant nematodes is spent feeding in the rhizosphere of the host plant. Thus, due to the abundance of fluorescent pseudomonads recorded in the rhizosphere community and to their ability to act as a natural biocontrol agent (Stirling, 1991), individuals belonging to the group have been studied to examine their effects on the plant-parasitic nematode populations and their control (Kluepfel *et al*., 1993; Westcott & Kluepful, 1993; Hackenberg *et al*., 2000; Kluepfel *et al*., 2002). Studies have shown that the amount of damage that is caused by plant-parasitic nematodes has been reduced through the use of *Pseudomonas* spp., *Streptomyces* spp., *Bacillus* spp., *Pasteuria penetrans* and a wide variety of fungal species (Becker *et al*., 1988; Stirling, 1991; Meyer, 2003).

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In a few cases, *Pseudomonas* sp. (BG33R) was found to subdue the reproduction of ring nematodes under greenhouse conditions and to stop *in vitro* egg hatching (Kluepfel *et al*., 1993; Westcott & Kluepfel, 1993). Conversely, second-stage juveniles and adults have been seen to be unaffected by the presence of the bacteria strain for up to a period of two weeks. Although the use of BG33R in combination with soil solarisation resulted in a visible decrease in *C. xenoplax* numbers, repeated applications had to be implemented to maintain an optimum population of the biological control agent (Kluepfel *et al*., 2002). Bacteria use a variety of mechanisms to stop nematode egg hatching. The mechanisms used include the production of: toxins; antibiotics; enzymes that are lipolytic, chitinolytic and proteolytic; and such toxic compounds as cyanide, ammonia and hydrogen sulphide (Siddiqui & Mahmood, 1999).

A number of studies have shown the effectiveness of bacteria as a nematode control strategy. However, most of the studies conducted were carried out under controlled conditions using sterilised soil. Such experiments should be repeated under field conditions, so as to determine the effects under more natural conditions (Siddiqui & Mahmood, 1999), as well as to improve the knowledge of mechanisms used by bacteria to control nematode populations (Westcott & Kluepfel, 1993).

Entomopathogenic nematodes

Populations of plant-parasitic nematodes associated with roots and soil have been shown in some research, to be reduced by means of the use of entomopathogenic nematodes. Grewal *et al*. (1997) reported that *Mesocriconema* spp. were suppressed by means of the employment of *Steinernema riobrave* Cabanillas, Poinar & Raulston, 1994 on grass. In carrying out a project to record the effects of *S. riobrave and Heterorhabditis bacteriophora* Poinar, 1975 under greenhouse conditions, Nyczepir *et al*. (2004) found that, no effect on the *C. xenoplax* populations present.

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As seen above, no single management strategy is, as yet, effective in reducing tree loss, leading to a combination of elements being required to provide optimum conditions for plants to persist and maintain their production levels (Zehr *et al.*, 1976).

FUNGI

Dead *C. xenoplax* have been reported as being associated with *Hirsutella rhossiliensis* Jaffee & Zehr (1983) went on to test the latter's effects on *C. xenoplax* under laboratory conditions, documenting the presence of *H. rhossiliensis* in 20 of the 23 orchards sampled. Symptoms of the infection of *C. xenoplax* by the fungus include the presence of a distorted body and the browning of the head region, together with the presence of hyphae carrying *H. rhossiliensis* phialides. Although the study concerned served to verify that *H. rhossiliensis* is definitely a parasite of both adult and juvenile *C. xenoplax*, the knowledge of effects on the nematode population is still lacking (Jaffee & Zehr, 1983).

A relatively high degree of parasitism by *H. rhossiliensis* was observed in adults, compared to that which was experienced in juveniles under field conditions, with the former being ascribed to the amount of stress that was brought on by a number of factors, including water, the presence of nematicides, and starvation. When temperature-stressed adults were inoculated with a fungus following exposure to a temperature of 40°C for 60 min, they were readily parasitised and killed within a period of 30 minutes (Jaffee & Zehr, 1983). The eggs of the *C. xenoplax* were not found to be infected by *H. rhossiliensis*, with them undergoing normal development, and hatching after approximately two weeks.

MANAGEMENT PRACTICES

The application of soil solarisation, crop rotation and fallow to control relatively low plant-parasitic nematode populations are not viable options for use with such perennial crops as grapevine and stone fruit. However, in South Africa the planting of annual cover crops between vine rows is a common soil cultivation practice in vineyards (Kruger *et al*., 2013). Benefits that can be gained from the use of cover crops include weed control, soil cover and biofumigation

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effects. However, such use could also have both a positive and a negative effect on such economically important nematodes as ring nematodes.

In a three-year study involving the planting and mechanical incorporation of the cover crop green biomass concerned into the soil of the work row in a vineyard, using cover crops with known biofumigation properties, was conducted in South Africa (Kruger *et al.*, 2013). The results showed a consistent reduction in the number of ring nematode in the vine row during four sampling periods, days after the mechanical incorporation of Canola, *Brassica napus* cv. AV Jade and Caliente, *Brassica juncea cv. Caliente 199* in the soil. The results obtained were mainly attributed to the host status of the cover crops in relation to the ring nematode (Kruger *et al.*, 2015b), rather than due to their biofumigation properties. In contrast, the use of White mustard*, Sinapis alba* showed a constant increase in ring nematode numbers in the vine row over the three-year period, making it unsuitable as a future cover crop for grapevine and stone fruit (Kruger *et al.*, 2015a).

CONCLUSIONS

As the ring nematode, *C. xenoplax* is a key pest in stone fruit orchards and vineyards worldwide, the development and improvement of alternative control methods to minimise damage is of critical importance. However, little is still known about many aspects of the nematode, leading to a requirement for much research still to be completed in this regard. Although a number of control methods have already been tested and used in the field, none has yet proved to be as effective in controlling nematode populations as has chemical control. The development of sound knowledge of the ring nematode is thus crucial to improving the implementation of such alternative control methods as the use of resistant rootstocks and of biological agents, to facilitate the movement away from the use of nematicides in the field.

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CHAPTER 2

DISTRIBUTION OF RING NEMATODE SPECIES IN THE WESTERN AND NORTHERN CAPE PROVINCES, AND THEIR MORPHOLOGICAL AND MOLECULAR CHARACTERISATION

ABSTRACT

The ring nematode, *Criconemoides xenoplax* (Raski, 1952) Loof & De Grisse, has been found to be a key economic pest in South African vineyards and stone fruit orchards. A survey was conducted in both vineyards and orchards in the Western and the Northern Cape provinces to determine the distribution, and to obtain specimens, of ring nematodes from the different production areas. Molecular and morphometric characterisation was used to identify the ring nematode species present. A total of 49 soil samples were taken from randomly selected farms. Additional data from the diagnostic laboratory, Nemlab (R44 & Anyswortel Rd, Klapmuts, Paarl, 7625), were analysed with regards to the occurrence of ring nematode in soil samples obtained from the stone fruit orchards and vineyards soil samples. Analysis of the morphology and the ITS region of ring nematodes concluded that *C. xenoplax* was the only ring nematode species to be retrieved from the stone fruit and grapevine samples, except for one site that had an unknown species present, confirming its broad-based distribution within the area. A 100% occurrence of *C. xenoplax* was recorded in all the samples analysed during the survey, ranging from as few as 20 to >2000 nematodes per 250 ml of soil. The infestation of *C. xenoplax* followed similar trends observed worldwide, substantiating its status as an economically significant pest. The need for alternative management options for the control of the nematode concerned is essential for the sustained health of stone fruit and grapevine production.

Key words**:** *Criconemoides xenoplax,* distribution, identification, Northern Cape, ring nematode, stone fruit, vineyards, Western Cape

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INTRODUCTION

The first report of *Criconemoides xenoplax,* (Raski, 1952) Loof & De Grisse, 1967, from South Africa was made by Heyns in 1970. The ring nematodes are seen as a significant economic pest within the group of plant-parasitic nematodes (Heyns, 1970b). The group of nematodes concerned consists of a number of cosmopolitan species, with several of the species occurring in South Africa. The distribution of some of the species in the ring nematode group is described as local, with others being indigenous in nature. Ring nematodes in South Africa are found in a diverse range of vegetation types, including orchards, vineyards, forests, gardens, plantations, and field crops, in addition to pristine veld (Heyns, 1970b).

Plant-parasitic nematodes have a high diversity in the rhizosphere of grapevine. The above could be a result of introduction by infested plant material, or the nematodes could have been already present in the soil, prior to the establishment of the vineyards or orchards concerned. Thus, nematode communities in the soil are shaped by the preceding crop or plant community. Meyer (1999) found that the initial introduction of such nematodes as *C. xenoplax* could be by way of fynbos, which acts as a source of inoculum. In monoculture, specific nematode populations can attain high numbers, due to the presence of a favourable host, such as grapevine.

Criconemoides xenoplax is commonly found in vineyards in the Western Cape province. In 1987, Pieterse & Meyer conducted a study on the distribution of *C. xenoplax* in the Western Cape province. The survey concluded that 90% of the established vineyards and over half of the vine nurseries were infested with *C. xenoplax*. Also, Marais & Swart (2002) conducted a survey as part of the South African Plant Parasitic Nematode Survey Database (SAPPNS). They recorded the presence of *C. xenoplax* in the Northern Cape province, and in the Modimolle area of the Limpopo province. Its abundance in the Northern Cape was 67% and 75%, in vineyards and orchards, respectively. In the Modimolle area, it was also recorded to be associated with grapevines and orchards, in relation to which it was found to have an

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incidence level of 32%. In 1998, Marais and Swart also recorded the occurrence of *C. xenoplax* in the Western Cape on four different fruit hosts, as well as on a wide variety of plants.

Raski, in 1952, initially described *C. xenoplax,* which belongs to the family Criconematidae, from around the roots of Thompson seedless grapes from a sample obtained near Fresno, California. Since its discovery, *C. xenoplax* has been recorded to have a worldwide distribution in regions of Japan (Core, 2001), North and South America, Australia, Africa, Europe (Weischer, 1960), and India (De Grisse, 1968). *Criconemoides xenoplax* is the most widely distributed species of the genus *Criconemoides* (Seshadri, 1964).

Considerable revision of the Criconematids taxonomy started in the 1960s. However, much uncertainty and controversy has existed regarding its genus legitimacy, due to the independent and simultaneous revision of the group's taxonomy, the description of which is being undertaken by a number of researchers in different parts of the world. Despite the availability of many studies concerning the morphology of *Criconemoides* that are available, insufficient data are available on the *C. xenoplax* taxonomy (Subbotin *et al*., 2005). The proper management of ring nematodes depends on the correct nematode identification, due to their parasitism of economically important crops (Cordero *et al*., 2012). Understanding the plant parasitism phenomenon, thus, hinges on the development of the morphological and molecular status of different *C. xenoplax* populations from various geographical regions (Subbotin *et al*., 2005).

De Ley *et al*. (2005) analysed the internal transcribed spacer (ITS), and the D2 and D3 regions of *C. xenoplax* from two different areas in California. The existence of two variants was discovered, differing by eight substitutions in the ITS region. The finding was supported by means of the morphological differences detected in the length of the stylet.

Despite the intensive study of the morphological taxonomy of the Criconematids, no consensus, as yet, exists about the validity of the genera and the proposed species in the ring

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nematode group, because of the varied interpretations of morphological characters by taxonomists. Currently, however, molecular techniques, in combination with traditional morphological methods, are providing an accurate and reliable method of identification of the species within a specific group of ring nematodes (Subbotin *et al*., 2005).

In 2005, Subbotin *et al*. were the first researchers to use DNA sequence data to study the phylogeny of the ring nematode group. Their phylogenetic analysis was based on the D2 to D3 expansion segments of the 28S rRNA with the DNA fragment. The incorporation of mitochondrial DNA (COI and COII) was also suggested as aiding in understanding the phylogenetic relationships involved (Cordero *et al*., 2012).

The objectives of the current study were to conduct a survey on the occurrence of ring nematode from grapevine- and stone-fruit-producing areas in the Western and Northern Cape provinces. After extracting the nematodes, samples were analysed using morphometrics and molecular characterisation to identify the ring nematode concerned to species level. Soil samples analysed by a diagnostic laboratory for the presence of nematodes from vineyards and stone fruit orchards during 2015 were mined for the occurrence of ring nematode, with regard to the origin and number of nematodes present in the samples.

MATERIALS AND METHODS

RING NEMATODE SURVEY

A survey was carried out to determine the distribution of *C. xenoplax* in soils obtained from randomly selected stone fruit orchards and vineyards throughout the Western and Northern Cape provinces (Fig.2.1). A single block was selected for sampling per farm. The selected block was then divided into four quadrates, and a total of five randomly selected trees/vines being sampled in each quadrate, with a total of 20 trees/vines being sampled at each site.

Soil was collected 30 cm away from the base of the tree, at a depth of approximately 20 to 30 cm. Approximately 1 kg of soil was collected at each sampling site. Sampling was carried out

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in spring and summer. Each soil sample was placed in a plastic bag and labelled with the relevant information (i.e. rootstock, sample date, fruit type, cultivar, and sampling area). The samples were then placed in a cool box, and transported to the laboratory for further analysis.

FIGURE 2.1. A. Sampling areas in the Western Cape province. B. Sampling areas in the Northern Cape province.

NEMATODE EXTRACTION

Nematodes were extracted using the sugar centrifugation and flotation method developed by Jenkins (1964), which relies on the specific gravity of nematodes to separate them from soil and organic debris. After using water in the centrifugation process a suspension of organic matter with a specific gravity of <1 g cm−3 will stay and can be thrown out. The process allows for the rapid extraction of both living and dead nematodes from a sample (Marais *et al*., 2017). Afterwards, centrifugation in a sugar solution allows the nematodes to remain in suspension. The suspension containing the nematodes were then transferred to a 38-µm – aperture sieve and the nematodes retained on the sieve, were washed into a beaker containing distilled water

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for further analysis. Minimising the exposure of the nematodes to the sugar solution, to decrease and prevent osmotic stress, is important (Marais *et al*., 2017)

NEMATODE ENUMERATION

The nematode samples collected were decanted into a beaker and left for 24 h, for the nematodes to settle to the bottom of the suspension. Water was then siphoned off using a rubber tube to a level of 20 ml. After mixing of the sample by aeration using a fish pump, two 1-ml aliquots was drawn from the sample using a pipette. The aliquots was placed separately onto a Peter's slide, and the number of ring nematodes per slide was counted. The average of the two counts was multiplied by 20 to estimate the number of nematodes per 250 ml soil.

CHARACTERISATION OF C. XENOPLAX

MORPHOLOGY

A total of 30 to 40 ring nematodes per sample were picked from each sample, with the aid of a dissection microscope and a pin vice handling tool. The nematodes were then placed in a glass cavity block containing a small amount of distilled water. To prepare the permanent slides, the Seinhorst's rapid technique (Seinhorst,1959) was used. To fixate the nematodes, water was removed using a syringe, and it was replaced with heated FAA solution (6 ml 40% formaldehyde, 1 ml acetic acid, 20 ml 96% ethanol, and 40 ml distilled water) (80°C). The container was then placed in a glass Petri dish, where it was kept at room temperature for a period of 3 to 4 days. The nematodes were then further processed to 100% glycerol. The processing was done by means of removing the FAA and replacing it with Seinhorst I (20 parts of 96% ethanol, 79 parts distilled water, and one part glycerol). The cavity block was transferred to a small desiccator with alcohol in the bottom, which was maintained at a temperature of 45°C for 2 to 3 days for the water in the nematodes to be replaced by alcohol. The specimens were then submerged in Seinhorst II (5 parts glycerol, and 95 parts 96% ethanol), and the cavity block was then transferred back into a glass Petri dish, and placed in the oven to replace the alcohol in the nematodes with glycerol. Once the process was

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complete, the nematodes were ready for permanent mounting on glass slides. This was done by placing a drop of dehydrated glycerol in the centre of the slide, whereupon five nematodes were transferred to the drop, and lightly pressed down to the bottom of the drop. The nematodes were lined up close to each other in one direction, and a coverslip was placed in position. The prepared slide was placed on a hot plate to allow the paraffin wax rings to melt. Once melted, the edges of the coverslip were sealed using glyceel, and the slide was labelled. After examining the nematodes from each sample under the microscope, they were characterised both morphologically and morphometrically. For morphometrical measurements, a Zeiss research microscope (Leica DM2000) fitted with a camera (Leica DFC295), a computer, and LAS 4.0 live measuring software was used.

MOLECULAR ANALYSIS

Ten individual nematodes were picked from each of the samples, with them being individually used for the molecular analysis. The nematodes were individually placed in 10 µℓ lysis buffer (50 mM MgCl2, 10 mM DTT, 4.5% Tween-20, 0.1% gelatine, and 1 µℓ proteinase K, at 60 µg m-1) on the side of an Eppendorf tube, after which each nematode was cut into pieces with the aid of the sharp side of a sterile insulin needle. The Eppendorf tube was then placed at -80°C for a minimum period of 15 min. For the DNA extraction, the tubes were then incubated at 65°C for 1 h, and at 95°C for 10 min, in order to lyse the cells completely, as well as to digest the proteins. The tube was cooled on ice and centrifuged at 11 600 g at 10°C for 2 min. The DNA was then collected and stored at -20°C for further PCR.

The PCR primers used to amplify the ITS rDNA region, including the ITS1, 5.8S and ITS2 ribosomal genes, as well as short parts of the 18S and 28S ribosomal regions, were TW81 (5'- GTTTCCGTAGGTGAACCTGC-3') and AB28: 5'- TATGCTTAAGTTCAGCGGGT-3 (reverse) (Hominick *et al*., 1997). The final reaction volume was 25 µℓ. The cycling conditions were as follows: denaturation at 94°C for 20 sec, annealing at 50°C to 55°C for 30 sec, with an extension at 72°C for 45 sec, with all conditions being repeated for 35 cycles. A 2-min incubation period at 72°C followed the last cycle, to complete any partially synthesised

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strands. The PCR product was then run on 1.5% agarose gel in a 1 × TBE buffer, and visualised by means of ethidium bromide staining.

The post-PCR purification was undertaken using the NucleoFast Purification System (Macherey-Nagel, Waltham. Massachusetts, USA). Sequencing was performed with the BigDye Terminator V1.3 sequencing kit (Applied Biosystems), followed by electrophoresis on the 3730 × 1 DNA Analyser (Applied Biosystems, Foster City, California, USA) at the DNA Sequencing Unit (Central Analytical Facilities, Stellenbosch University). The forward and reverse sequences generated from the ITS region of the 18S rDNA gene were aligned using CLC Main Workbench (ver. 7.3.3), and compared with sequences available on the GenBank (NCBI). Further alignment was done using ClustalX 2.1 (Thompson *et al*., 1997). The distance analysis of closely related sequences was conducted in MEGA5 (Tamura *et al*., 2011).

Analysis of diagnostic samples

Results from the stone fruit and grapevine samples analysed during 2015, by a private diagnostic nematode laboratory, Nemlab, near Klapmuts in the Western Cape province were mined for data regarding the distribution and density of the ring nematodes in the different production areas. The nematodes were extracted by Nemlab from the soil samples using the same technique (Jenkins, 1964) as was used for all the extractions in the current study.

STATISTICAL ANALYSIS

The data obtained from the survey and the diagnostic laboratory Nemlab were analysed by means of a one-way ANOVA detect the variances in *C. xenoplax* numbers recorded in the different production areas sampled. The different characteristics measured were analysed using STATISTICA (ver.13.2.).

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RESULTS

DISTRIBUTION OF C. XENOPLAX

Analysis of 46 soil samples from stone fruit orchards and vineyards showed 100% infestation with *C. xenoplax*. The mean number of nematodes per sample was found to be 624 ± 145, with an overall significant difference between the production areas sampled ($F_{(5, 40)} = 6.9843$, $p < 0.05$). The high mean ring nematode numbers in 250 cm^3 soil, in Kakamas (2106 ± 265) and Blouputs (2106 ± 427) in the Northern Cape, differed significantly from the rest of the areas sampled. The ring nematode numbers in the other areas were generally lower (< 300), and did not differ significantly from one another (Fig. 2.2).

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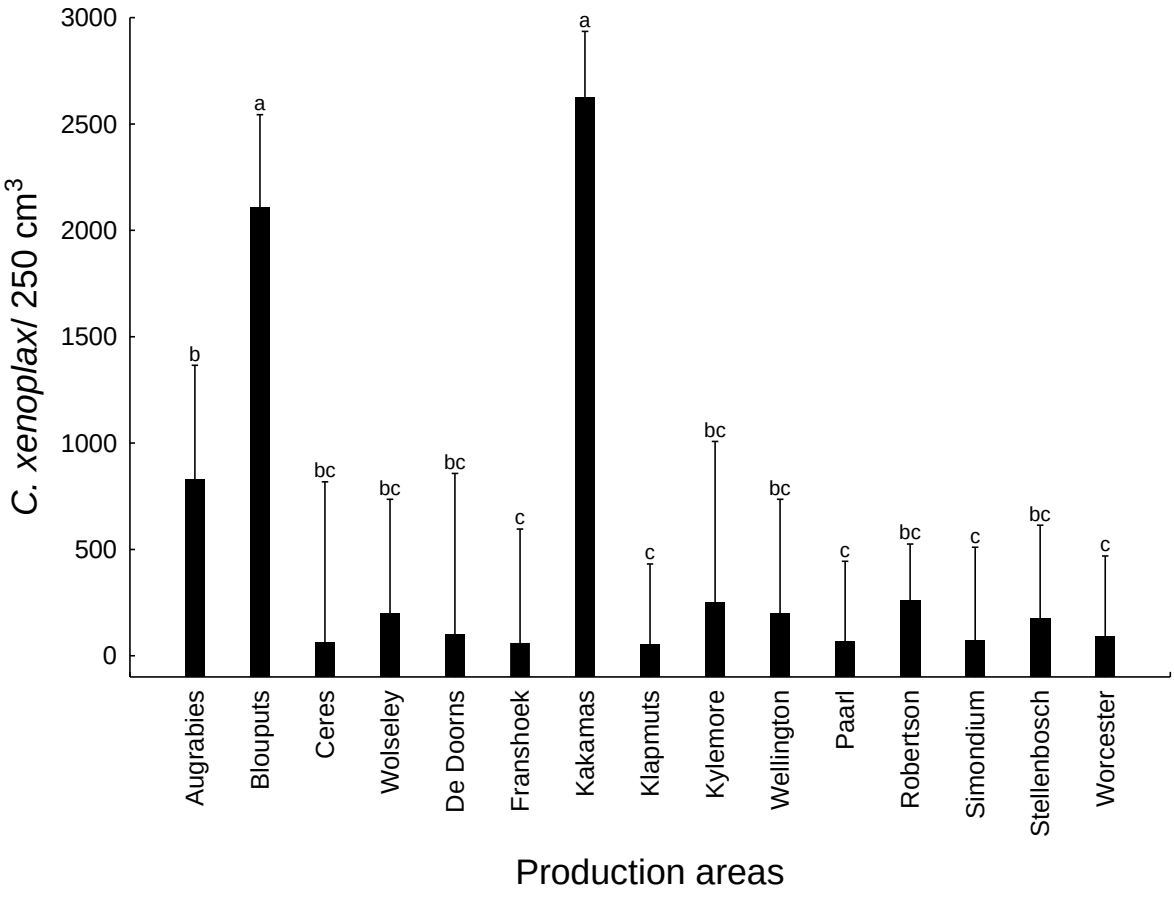

Figure 2.2. The mean number of *Criconemoides xenoplax* and its distribution within the different production areas ($F_{(5, 40)} = 6.9843$, $p < 0.01$). The same letter above the bars indicates no significant difference.

In terms of the results of the *C. xenoplax* numbers recovered from the different hosts sampled, the grapevine, peach, plum and apricot showed an overall difference ($F_{(3, 39)} = 10.858$, $p < 0.05$) between the samples. The number of ring nematodes observed on the grapevine (2627 ± 303) differed significantly from the number of such nematodes on plum (160 ± 43.8), peach and apricot. However, no differences were observed in the ring nematode numbers recorded between the latter three hosts (Fig. 2.3).

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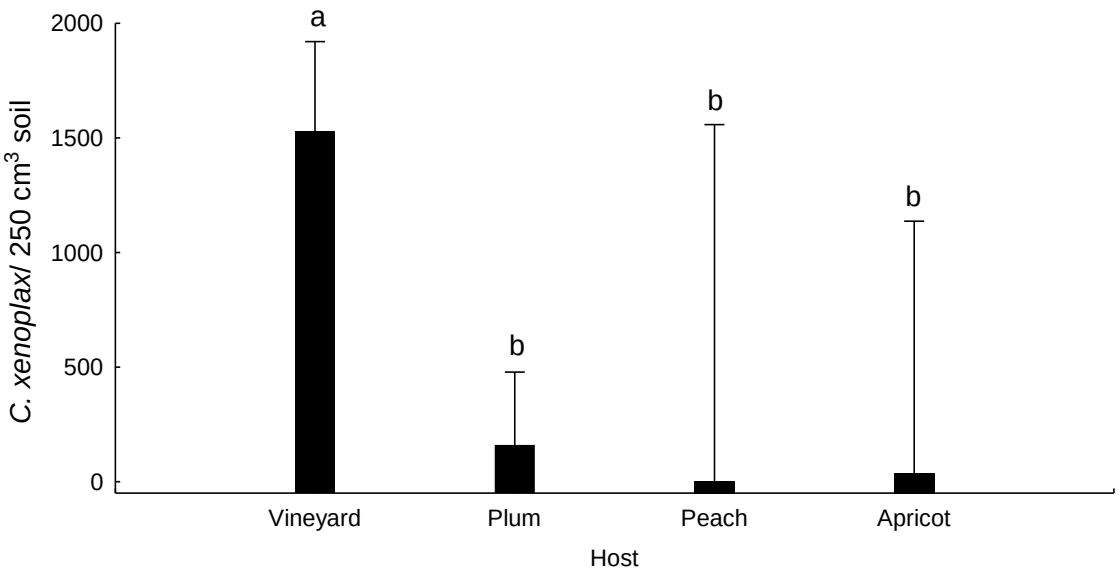

Figure 2.3. The mean number of *C. xenoplax* numbers recorded on the different hosts sampled in the Western Cape province ($F_{(3, 39)} = 10.858$, $p < 0.05$). The same letter above the bars indicates no significant difference.

ANALYSIS OF RESULTS FROM NEMLAB

A total of 1754 soil samples from stone fruit and grapevine were analysed by Nemlab. The results showed that the grapevine had the highest incidence of *C. xenoplax* infestation, with 1214 (69%) soil samples being infested, compared to the different stone fruit samples analysed. Peach had the second highest occurrence of *C. xenoplax* infestation, with a total of 288 (16%) samples being infested. Plum followed peach, with 237 (14%) samples being infested, while apricot had the lowest infestation rate of 15 (1%) plants (Fig. 2.4).

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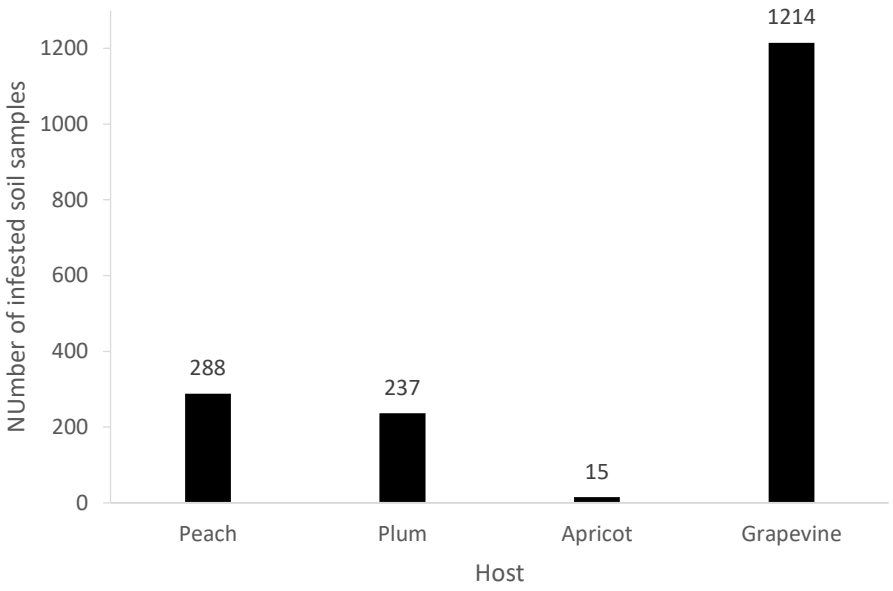

Figure 2.4. Number of samples per host infested with *Criconemoides xenoplax,* obtained from samples analysed from Nemlab during 2015.

The results concerning *C. xenoplax* numbers recorded on the different hosts obtained from the routine samples of Nemlab show that plum (506 ± 44) had the highest nematode populations recorded, which differed significantly ($p < 0.05$) from grapevine (358 ± 16) and peach (260 ± 27). The latter two hosts also had a significant difference in the *C. xenoplax* populations recorded ($p = 0.01$). The nematode numbers recorded on apricot (303 ± 112) showed no differences when compared to the other hosts, despite there being extensive variation observed in the nematode numbers between the apricot plants sampled (Fig. 2.5).

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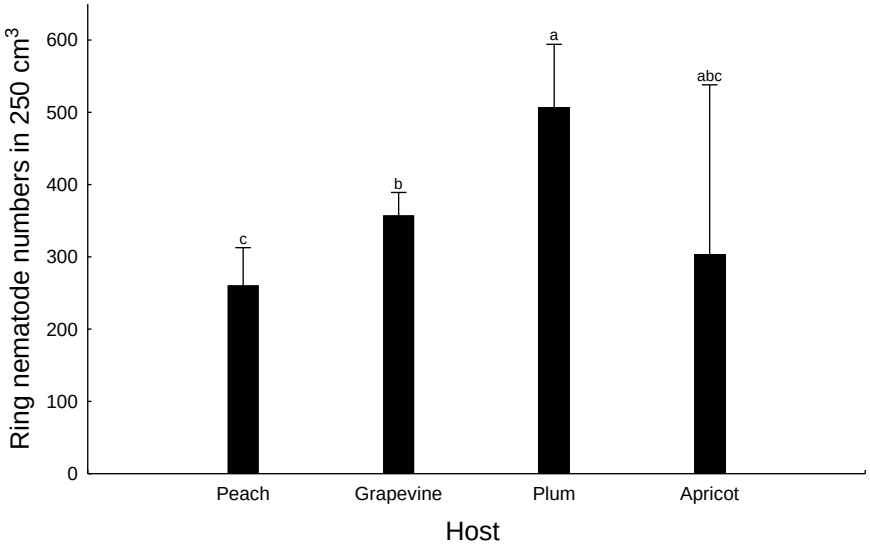

Figure 2.5. The mean number of *Criconemoides xenoplax* numbers recorded on the different hosts sampled in the Western Cape region, obtained from routine soil samples analysed by Nemlab for 2015 (one-way ANOVA, $F_{(3, 2293)} = 8.4093$, $p < 0.05$). The same letter above the bars indicates no significant difference.

An overall significant difference was observed ($F_{(6, 1389)} = 17.274$, $p < 0.005$) in the numbers of *C. xenoplax* recorded between the occurrence of ring nematodes in grapevine in the areas sampled. Worcester (745 ± 88.97) had the highest nematode population compared to the remaining areas, although it did not differ significantly ($p > 0.05$) from Robertson (427 ± 61.25) and the Olifants River (459 ± 41.26). The *C. xenoplax* populations recorded in Paarl (214 ± 28.04), the Swartland (247 ± 19.86) and Hex River (267 ± 26.68) were relatively similar. A substantial difference ($p < 0.05$) was recorded in the *C. xenoplax* populations observed in Tulbagh (66 ± 19.57), which had the lowest nematode counts when compared to the other areas sampled (Fig. 2.6).

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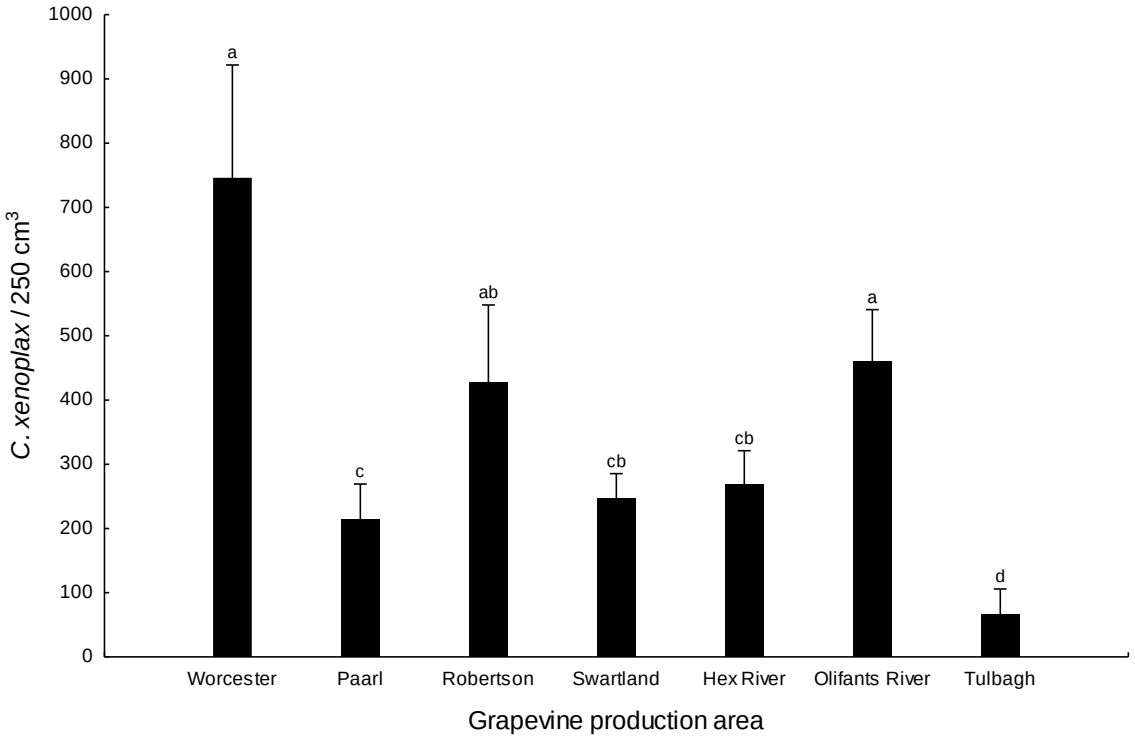

Figure 2.6. Results of the mean number of *Criconemoides xenoplax* and its distribution within the grapevine production areas, obtained from soil samples analysed by Nemlab from January to December 2015 ($F_{(6, 1389)} = 17.274$, $p < 0.005$). The same letter above the bars indicates no significant difference.

MORPHOMETRICS

No obvious differences in morphological characteristics were observed during the light microscopy analysis of the permanent slides of the ring nematodes from samples taken during the survey (Fig. 2.10). A one-way analysis of variance (ANOVA) was used to analyse each characteristic measured to account for any variation in the characteristics of *C. xenoplax* populations, within and between the different areas sampled. A significant difference was found in the characters measured between the *C. xenoplax* populations recorded from the different sampling areas, ($p < 0.005$). Little to extensive variation in the characteristics within the different populations was also recorded (Table 2.1). The lengths of *C. xenoplax* differed significantly ($F_{(8, 229)} = 15.077$, $p < 0.005$) between the different areas sampled. De Doorns had the smallest nematodes, with a mean length of 394 ± 38.5 µm, which differed significantly from

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the nematodes recovered from Augrabies, which had the largest nematodes, with a mean length of 631 ± 17.2 µm (Fig. 2.8).

The other characteristics measured were positively correlated to the length of the nematode, thus the larger the nematode, the longer the stylet and the oesophagus, and the wider the width. However, stylet length as a percentage to body length was greatest in the smaller nematodes recorded in De Doorns. However, the nematodes recovered from De Doorns had the most variation within the population, compared to the other areas sampled. An overall significant difference was observed in the stylet length as a percentage of the body length in all the populations recovered ($F_{(8, 229)} = 6.2243$, $p < 0.01$) (Fig. 2.7). Samples with fewer than 100 nematodes per 250 cm^3 soil were not characterised morphometrically. The DNA analysis was only done on the samples that lacked sufficient nematodes for both DNA and morphometric analysis.

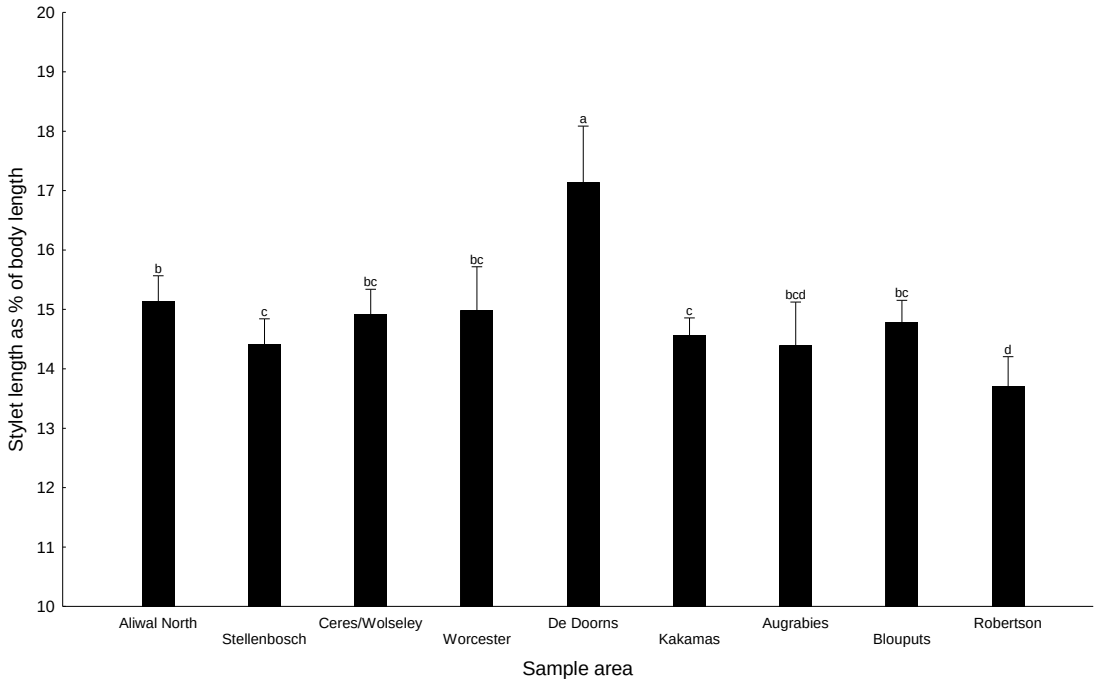

Figure 2.7. Mean stylet length as a percentage of the body length of *Criconemoides xenoplax* populations recorded from both the stone orchards and the grapevine areas sampled during the study ($F_{(8, 229)} = 6.2243$, $p < 0.01$). The same letter above the bars indicates no significant difference.

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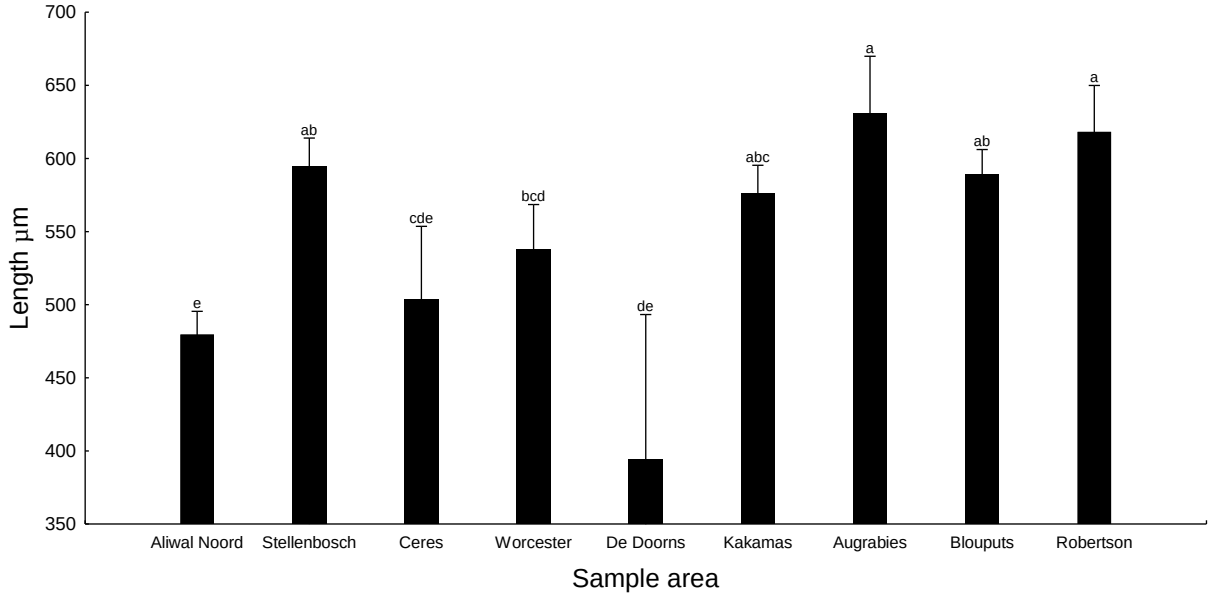

Figure 2.8. Mean lengths of *Criconemoides xenoplax* populations recorded from the both stone orchards and grapevine areas sampled during the study ($F_{(8, 229)}$ =15.077, p <0.005). The same letter above the bars indicates no significant difference.

DNA ANALYSIS

No variation in the DNA analysis of the ITS region was observed between the *C. xenoplax* populations collected from the different production areas sampled. However, in the case of isolate 2343 from pear samples from the farm De Hoop in the Ceres area, an unknown species of ring nematode was found. According to the Basic Local Alignment Search Tool (BLAST) tool on GenBank, the identity of the nematode differed from that of *C. xenoplax* by 82% with a 100% coverage, with its most closely related species being *Mesocriconema surinamense*. The consensus tree from the maximum parsimony bootstrap analysis for the ITS region of *Criconemoides* and *Mesocriconema surinamense* is shown in Figure 2.9.

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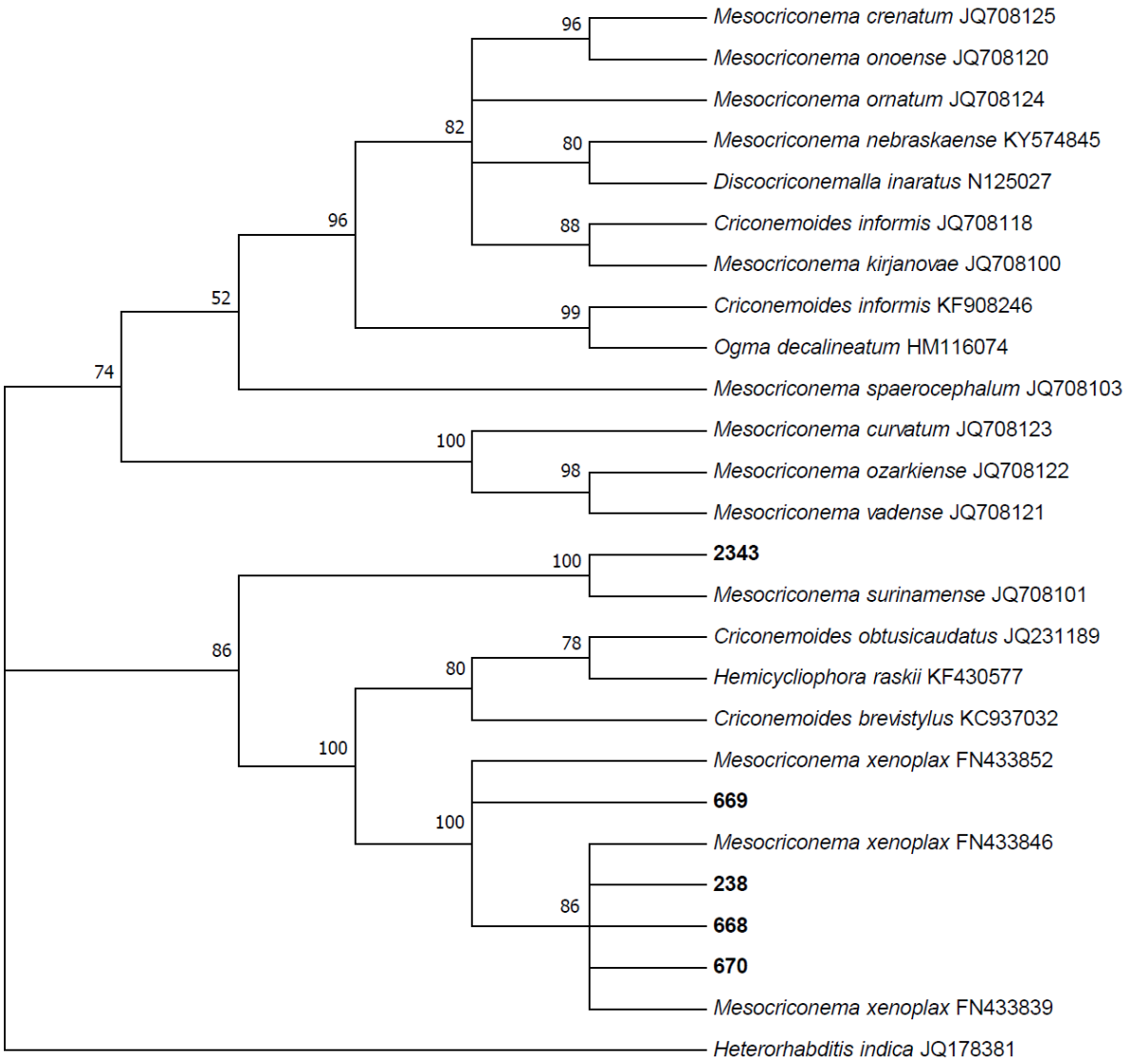

Figure 2.9. Consensus tree from the maximum parsimony bootstrap analysis for the ITS region of *Criconemoides* and *Mesocriconema*. Bootstrap replicate percentages are shown at the branch points that support the clades.

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TABLE 2.1. Morphometrics of *Criconemoides xenoplax* obtained during a survey of stone fruit orchards, vineyards and nut production areas. The mean, range and standard deviation are indicated in micron (µm).

Cape Region	Eastern		Northern		Western							
	Aliwal North	Augrabies	Blouputs	Kakamas	Stellenbosch		De Doorns	Robertson	Worcester	Ceres/ Wolseley		
Host	**Walnuts**	**Grapevine**	**Grapevine**	**Grapevine**	**Grapevine**	**Plum**	**Grapevine**	**Plum**	**Grapevine**	**Pears**	**Grapevine**	**Plum**
n	30	20	30	120	20	10	10	20	10	10	10	10
Body length	480 ± 41	602 ± 56	595 ± 53	576 ± 73	554 ± 39	616 ± 47	394 ± 86	623 ± 74	538 ± 41	333 ± 27.1	563 ± 35	614 ± 73
	(407-587)	(528-738)	(498-672)	(395-753)	(503-641)	(560-688)	(281-552)	(441-498)	(443-593)	(271-382)	(512-607)	(537-804)
Oesophagus length	130 ± 10.26	146 ± 9.52	144 ± 8.74	141 ± 13.03	150 ± 4.68	157 ± 10.04	117 ± 16.67	144 ± 9.55	141 ± 8.43	97.52 ± 5.34	147 ± 6.06	150 ± 6.06
	(115-156)	(129-168)	(126-171)	(110-166)	(139-159)	(142-172)	(89.15-146)	(123-160)	(129-155)	(83.32-103)	(136-156)	(144-164)
a	11.2 ± 0.5	11.44 ± 0.89	11.62 ± 0.79	11.57 ± 0.93	11.35 ± 0.68	12.1 ± 0.73	10.23 ± 0.79	12.26 ± 0.96	11.02 ± 0.63	9.67 ± 0.28	11.52 ± 0.42	12.33 ± 0.81
	(10.02-1221)	(9.98-13.1)	(9.92-13.13)	(9.65-13.61)	(10.25-12.66)	(10.62-13.46)	(9.22-11.73)	(11.08-14.96)	(10.1-12.03)	(9.22-10.39)	(11.08-12.52)	(11.42-14.27)
b	3.7 ± 0.2	4.11 ± 0.21	4.12 ± 0.24	4.08 ± 027	3.7 ± 0.21	3.91± 0.07	3.34 ± 0.31	4.31 ± 0.4	3.82 ± 0.4	3.42 ± 0.2	3.84 ± 0.13	4.07 ± 0.32
	(3.23-4.05)	(3.71-4.44)	(3.7-4.68)	(3.57-4.97)	(3.31-4.11)	(3.82-4)	(2.83-3.78)	(3.57-5.11)	(3.18-4.29)	(3.13-3.73)	(3.6-4.04)	(3.68-4.9)
Max. body width	42.7 ± 3.21	52.61 ± 2.28	51.12 ± 2.05	49.71 ± 3.62	48.91 ± 3.22	50.96 ± 3.11	38.17 ± 5.47	50.74 ± 3.32	48.85 ± 3.63	34.41 ± 2.12	48.89 ± 2.18	49.65 ± 2.76
	(36.36-50.87)	(48.83-56.61)	(47.95-55.23)	(38.52-56.14)	(43.02-56.97)	(45.87-54.97)	(30.46-47.11)	(39.74-56.48)	(42.24-56.63)	(29.36-37.65)	(45.97-52.42)	(46.55-56.38)
Lip/vulva	450 ± 39.6	564 ± 54.2	556 ± 50.82	540 ± 69.85	522 ± 38.28	580 ± 44.4	365 ± 82.22	586 ± 70.85)	499 ± 38.57	312 ± 26.73	527 ± 33.56	576 ± 69.96
	(379-554)	(493-56.61)	(462.19-630)	(367-707)	(473-610)	(526-648)	(258-516)	(417.09-758)	(410-554)	(252-360)	(482-573)	(504-757)
Lip/median bulb	88.9 ± 8.58	103 ± 4.26	101 ± 4.25	98.17 ± 9.1	104 ± 4.08	105 ± 4.55	78.28 ± 11.68	99.31 ± 6.97	95.62 ± 9.55	69.45 ± 3.88	101 ± 5.74	101 ± 4.84
	(71.33-109)	(95.06-112)	(92.85-111)	(60.9-101)	(96.5-114)	(97.69-112)	(60.9-101)	(82-108.85)	(70.68-105)	(58.94-72.42)	(88.94-109)	(92.58-108)
Stylet length	72.6 ± 8.17	88.22 ± 3.7	86.72 ± 3	66.16 ± 9.38	83.34 ± 7.55	88.04 ± 5.91	66.16 ± 9.38	84.09 ± 5.32	80.33 ± 4.98	54.92 ± 2.77	82.42 ± 4.8	82.66 ± 4.7
	(62.37-89.94)	(80.77-95.3)	(80.71-93.54)	(54.27-85.33)	(63.32-93.53)	(77.31-96.61)	(54.27-85.33)	(68.13-92.31)	(71.86-89.11)	(47.52-58.65)	(70.86-87.79)	(73.26-88.86)

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Cape	Eastern		Northern					Western				
Region	Aliwal North	Augrabies	Blouputs	Kakamas	Stellenbosch		De Doorns	Robertson	Worcester	Ceres/ Wolseley		
Host	Walnuts	Grapevine	Grapevine	Grapevine	Grapevine	Plum	Grapevine	Plum	Grapevine	Pears	Grapevine	Plum
Length of stylet shaft	69.4 ± 7.93	84.42 ± 3.53	82.93 ± 2.86	62.99 ± 8.82	79.62 ± 7.34	84.32 ± 6.05	62.99 ± 8.82	80.45 ± 5.2	76.15 ± 4.95	51.64 ± 2.79	78.24 ± 4.3	79.31 ± 4.71
	(59.3-86.16)	(77.71-90.98)	(76.61-89.65)	(51.73-80.88)	(60.49-89.85)	(73.09-92.78)	(51.73-80.88)	(65.7-88.87)	(67.39-83.04)	(44.59-55.63)	(67.79-82.11)	(69.66-85.16)
%Stylet length body length	15.1 ± 1.1	14.76 ± 1.07	14.68 ± 1.16	17.13 ± 2.03	14.56 ± 1.09	14.34 ± 0.94	17.13 ± 2.03	13.6 ± 1.11	14.98 ± 0.9	16.53 ± 0.76	14.64 ± 0.64	13.56 ± 0.92
	(13.64-18.64)	(12.66-16.68)	(12.64-16.81)	(14.84-20)	(11.28-17.46)	(12.65-16.31)	(14.84-20)	(11.57-15.46)	(13.64-16.59)	(15.37-17.55)	(13.83-16.08)	(11.05-14.4)
Body width at vulva	33.5 ± 2.68	41.36 ± 2.36	40.26 ± 1.87	29.90 ± 3.59	38.5 ± 2.77	39.38 ± 3.06	29.90 ± 3.59	39.68 ± 2.9	38.44 ± 3.54	24.93 ± 1.61	37.66 ± 2.66	38.72 ± 2.34
	(27.38-39.61)	(37.28-45.59)	(37.33-44.02)	(25.29-36.48)	(32.86-45.81)	(30.64-41.88)	(25.29-36.48)	(29.89-42.75)	(33.06-45.1)	(21.86-26.78)	(32.05-42.65)	(35.58-44.54)
Vulva to end of body	29.6 ± 3.27	37.53 ± 2.6	39.2 ± 3.7	36.35 ± 4.14	32.62 ± 2.22	35.43 ± 3.1	28.87 ± 4.3	37.21 ± 4.38	36.35 ± 2.77	22.18 ± 1.61	36.34 ± 3.03	37.99 ± 4.42
	(23-37.05)	(33.13-42.09)	(34.46-49.59)	(28.13-46.23)	(29.32-37.38)	(29.77-42.9)	(22.59-36.28)	(23.44-43.95)	(32.64-40.53)	(18.38-24.45)	(30.24-41.05)	(32.53-46.87)
VL/VB	0.9 ± 0.1	0.91 ±0.07	0.97 ± 0.08	0.94 ± 0.07	0.89 ± 0.05	0.90 ± 0.05	0.96 ± 0.04	0.94 ± 0.08)	0.95 ± 0.04	0.89 ± 0.05	0.96 ± 0.04	0.98 ± 0.1
	(0.73-1.04)	(0.73-1.01)	(0.87-1.26)	(0.08-1.11)	(0.08-0.97)	(0.86-1.02)	(0.89-1.03)	(0.78-1.09)	(0.88-0.99)	(0.82-0. 96)	(0.9-1.03)	(0.87-1.15)
R	87.8 ± 6.19	99.9 ± 3.18	99.73 ± 2.82	97.07 ± 5.18	98.4 ± 3.73	98.5 ± 6..3	92.33 ± 5.76	101 ± 3.49	99.5 ± 1..5	79.5 ± 3.01	96.7 ± 3.29	99 ± 1..55
	(81-101)	(95-106)	(94-105)	(84-110)	(93-107)	(89-110)	(84-99)	(95-109)	(98-103)	(76-84)	(92-103)	(96-102)

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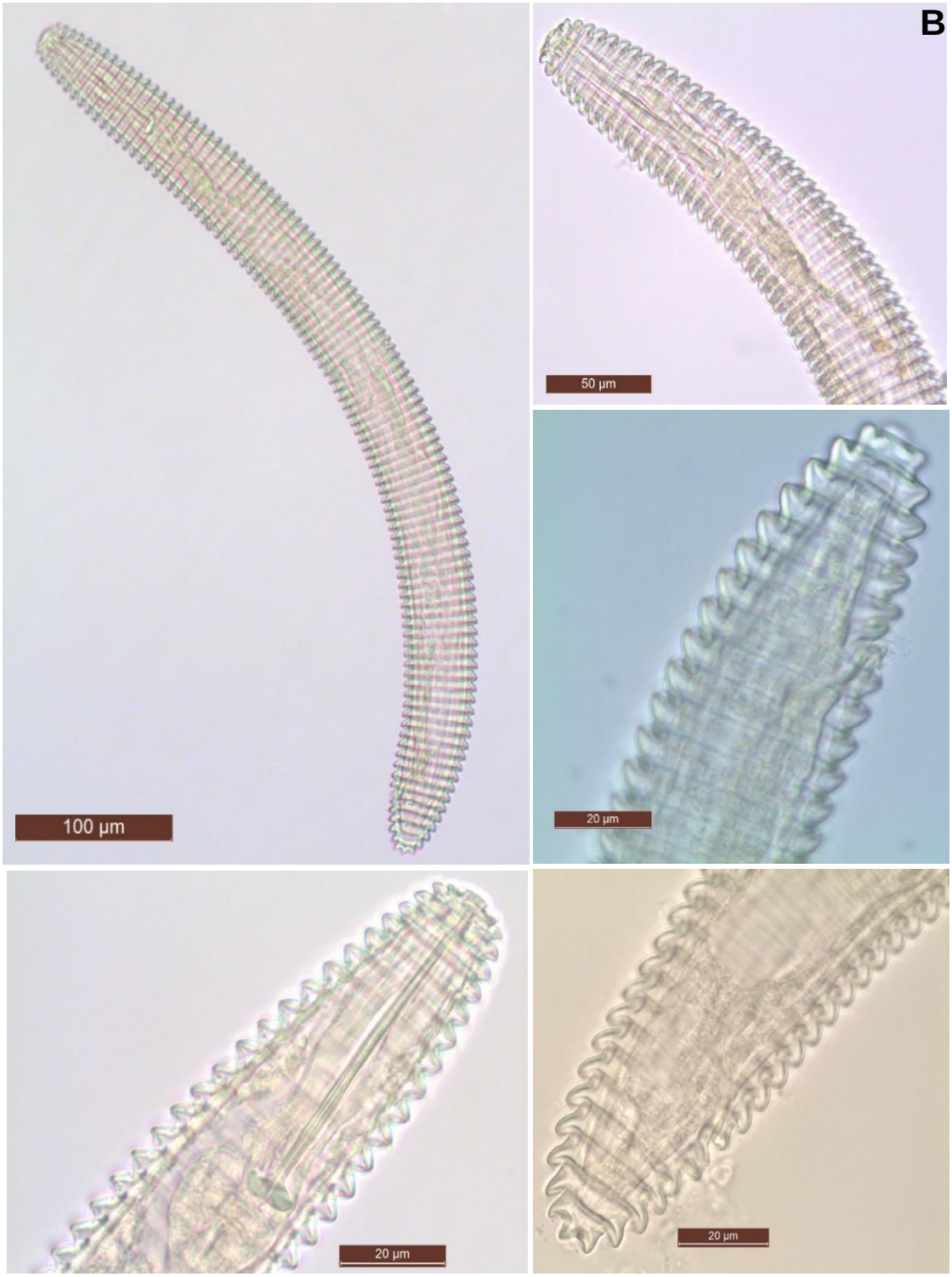

Figure 2.10. Photographs of *Criconemoides xenoplax*, as seen under a compound microscope. A. Entire length of female. B. Anterior portion showing oesophagus. C. Posterior end of body showing position of vulva. D. Anterior end showing stylet and lip region. E. Posterior portion showing tail shape. Scale bar in micron.

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DISCUSSION

Criconemoides xenoplax was the only species in the ring nematode complex recorded during the sampling of stone fruit orchards and vineyards in the Western and Northern Cape. The samples were taken from peach (*Prunus persica*), plum (*Prunus domestica*), apricot (*Prunus armeniaca*), and table and wine grapes. The occurrence and distribution of *C. xenoplax* in all the areas sampled during the study correlated with previous studies carried out in the Western Cape province. A total of 90% of the established vineyards (Pieterse & Meyer, 1987) and 40% to 50% of the stone fruit orchards were recorded as being infested with *C. xenoplax* (Marais & Swart 2001, 2002; Meyer, 1976). Keetch & Heyns (1982) describe *C. xenoplax* as being the most common ring nematode species occurring in orchards and vineyards in South Africa, while in 1973, Meyer concluded that *C. xenoplax* was the only ring nematode species to be present in all the samples collected. Similarly, studies conducted worldwide have reported the widespread occurrence and distribution of *C. xenoplax* (Seshadri, 1964) in grapevine (Pinochet & Cisneros, 1986; Weischer, 1960) and stone fruit orchards (Nyczepir *et al*., 1983, 1988).

During a survey conducted in 1970, Heyns recorded the presence of another nematode species belonging to the subfamily Criconematinae, *Macroposthonia curvatum* (Raski, 1952) De Grisse & Loof, 1965. However, only two specimens were recorded, one around the roots of a guava tree in the Paarl region, and the other recovered from a vineyard located in De Doorns. Later in 1998, Marais and Swart conducted a study as part of the South African Plant Parasitic Survey Programme (SAPPNS), recording the presence of *M. curvatum* from grapevine.

The densities recorded of nematodes in the areas sampled differed from highly severe (> 1000) to very low (<20) number of nematodes per 250 cm^3, with a number of the diagnostic laboratories (Nemlab, ARC diagnostic laboratory and Nemconsult) also reporting an increased occurrence of the ring nematode in the respective production areas. The samples brought in

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from the various production areas for routine analysis by Nemlab have also indicated increased occurrence of nematodes in the production areas, especially in the grapevine areas (Figure 2.1). The differences in population densities observed could be ascribed to a number of factors, including the rootstock used, the soil type, the soil moisture, the cultural practices employed, and the temperature. McKenry (1992) recorded a reduction in grape yield by 10% to 25% when the *C. xenoplax* populations exceeded 500 individuals per kg of soil, while Pinkerton *et al*. (2005) reported a reduction in yield when *C. xenoplax* numbers exceeded 125 per 250 cm^3. However, the effect, or the tolerable density, of *C. xenoplax* varies from one area to another, depending on the differences in soil characteristics, geographical areas, and management practices (Pinkerton *et al*., 2005).

The presence of *C. xenoplax* in all the production areas analysed could be because of the occurrence of natural vegetation and alternative hosts, as Meyer (1999) reported the persistence of nematode communities, such as *C. xenoplax*, on fynbos, which is regarded as the natural vegetation type in the Western Cape area. One plant species, *Protea repens,* was recorded to harbour such plant-parasitic nematode species as *Meloidogyne javanica, Scutellonema brachyurus*, and *C. xenoplax*. Thus, the production areas established on land formerly covered by fynbos tend to increase nematode populations, such as those of *C. xenoplax* (Meyer, 1999).

The morphological characteristics measured and observed between the *C. xenoplax* samples collected during the study varied among the different areas, from 394 ± 86.16 µm to 670 ± 38.65 µm (Table 2.1). Other studies carried out by Thomas (1959) and Heyns (1970a) showed variations in length of the nematodes measured, together with a number of other characteristics, like stylet length, ring numbers and others. Some of the morphological characteristics routinely used to identify the ring nematodes were not measured, because such characteristics as the anus not being visible.

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Research into the molecular identification of ring nematodes is scant, with very few sequence details being available on GenBank. The problems experienced with the constant change in the genus of ring nematode over the years, from *Criconema*, *Criconemella*, *Macroposthonia* to the current *Criconemoides,* exhibited problems with their morphological and molecular identification. However, during the survey, an unknown species that was closest related to *Mesocriconema* species, and which was not closely related to *C. xenoplax*, was found. However, no morphological differences were observed, except for the females being shorter in length compared to the other ring nematode samples collected.

The extensive distribution and high densities of *C. xenoplax* recorded in both stone fruit and vineyards in the Western Cape make control of the nematode a priority. Control of the ring nematode, *C. xenoplax*, is crucial to safeguard and maintain the health of stone fruit and vineyards throughout the Western Cape. However, before control of the nematode is implemented, it is important to identify the ring nematode species correctly, as ring nematode biology and plant-nematode interaction can vary (Nyczepir *et al.,* 1988). The management of *C. xenoplax* is very difficult and expensive, and it is usually a long-term commitment. The use of nematicides and other chemicals has been the main method of control in the past (Okie *et al.,* 1987; Storey *et al*., 2017). However, due to the industry's movement towards more sustainable management practices, and the restrictions placed on chemicals and their use, such alternative management options as resistant rootstocks, cover cropping, biological control, and crop rotation are being investigated. Other problems associated with the control of the ring nematode include the lack of virgin land, thus replanting on a particular site has become common practice. As a result, the number of nematodes has increased over the years (Hugo & Storey, 2017).

For future analyses, more data from private companies like Nemlab should be analysed, as doing so would enable the trends in *C. xenoplax* densities and distribution throughout grapevine and stone fruit orchards to be recorded. Thus, valuable information could be gained and used to aid in the implementation of management strategies, and in the prevention of

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build-up of ring nematode in the respective production areas. To obtain a more accurate reading of the nematodes present in the soil, samples should preferably be taken during the summer months, when the soil moisture and temperature are at favourable levels in the Western Cape province.

Sampling should also be uniform throughout the year, to enable the recording of a more accurate nematode reading, thus, other aspects such as soil type, before or after irrigation, and depth of sampling should be noted. A record of damage caused by the ring nematode should also be kept, to determine the duration of time required for the effects of feeding by *C. xenoplax* to become evident. The above would be especially important in the case of vineyards, as there is still some uncertainty as to whether or not *C. xenoplax* has an effect on grapevine yield and growth.

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CHAPTER 3

SUSCEPTABILITY OF COMMERCIAL GRAPEVINE ROOTSTOCKS TO THE RING NEMATODE, *CRICONEMOIDES XENOPLAX*

ABSTRACT

The ring nematode, *Criconemoides xenoplax*, is currently the main plant-parasitic nematode problem of grapevine in South Africa. Six commercial grapevine rootstocks were evaluated in a glasshouse for susceptibility to *C. xenoplax,* in a pot trial. Additionally, results from routine grapevine soil samples, from a diagnostic nematode laboratory, were analysed with regard to the number of ring nematodes extracted. The susceptibility of the different rootstocks was evaluated by means of using the nematode numbers and the reproductive factor for ring nematode. Results indicate that the overall C*. xenoplax* numbers from the five rootstocks differed significantly in the glasshouse trial. The rootstock, 110 Richter, had the highest number of *C. xenoplax* after 6 months, in both trials. Although *Criconemoides xenoplax* numbers on the rootstocks 1103 Paulsen and 140 Ruggeri did not differ in both trials, nematode numbers on 99 Richter and Ramsey were found to differ between the two trial dates. Despite the different grapevine rootstocks from the diagnostic samples obtained from Nemlab not differing in susceptibility, the number of *C. xenoplax* recorded was generally higher from the respective grapevine rootstocks from the routine samples from vineyards.

Key words: *Criconemoides xenoplax*, ring nematode, rootstock, vineyard, resistance

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INTRODUCTION

In South Africa, the practice of viticulture can be traced back to 1655, when the first vines were planted, in Cape Town, by Jan van Riebeeck. The South African grapevine production area covers a total area of 117 675 hectares, of which 99 463 ha are used for the production of wine, and 18 212 ha for the production of table grapes. Although the grapevine industry is mainly located in the Western Cape region, which comprises a total of 90% of production, the cultivation of grapevine has a widespread distribution, covering a variety of climates and soils (S.G. Storey *et al.*, 2017). Local grape vineyards have a number of key nematode pests recorded, including *Meloidogyne* spp., lesion, dagger and ring nematodes. In the survey conducted, the ring nematode *Criconemoides xenoplax* was observed in 48% of the samples analysed, with it being regarded as a major pest in Western Cape vineyards (Smith, 1977).

The ring nematode, *Criconemoides xenoplax* as well as numerous other species in the suborder, have been recorded as significant pests in agricultural production areas (Subbotin *et al*., 2005). Although the species are mainly associated with woody perennials, they have been recorded as being associated with several other plant species. *Criconemoides xenoplax* belongs to the family Criconematidea, as was originally described by Raski in 1952. Since its discovery, it has been documented as having a widespread distribution, as well as being regarded as the most common species recorded within the genus *Criconemoides* (Seshadri, 1964).

Nyczepir *et al*. (1983) were the first researchers to identify the association of *C. xenoplax* with the peach tree short life syndrome (PTSL). Since then, it has been implicated as a key predisposing agent that is responsible for the presence of the disease in stone fruit orchards (Ritchie & Clayton, 1981; Nyczepir *et al*., 1983), with it also being described as a significant pest in vineyards (McKenry *et al*., 2004; Pinkerton *et al.*, 2005).

The current and past management of *C. xenoplax* is achieved through the use of chemical nematicides, such as dibromochloropropane (DBCP) and ethylene dibromide (EDB) (S.G.

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Storey *et al.*, 2017). However, their use has been restricted as the result of negative effects recorded on both human and environmental health (Oka *et al*., 2000; Perry & Moens, 2006). Consumer demand has also resulted in a campaign for safer food supplies that have no, or only a limited, concentration of chemical residue and a restricted negative impact on the environment (Fourie *et al.*, 2017). The development and breakthroughs in biotechnology have also resulted in a shift to alternative methods of control. This is owed to the identification of genes that demonstrate resistance, as well as to the rapid identification, and development, of resistant varieties (Fourie *et al.*, 2017).

The high numbers of ring nematode populations observed in stone fruit and vineyard production areas (S.G Storey, pers. comm.), and the restricted use of chemical compounds, has resulted in the need to search for a more sustainable, or non-chemical, method of control by means of which to reduce ring nematode populations. Thus, research in the area of endeavour have focused on the increased implementation of alternative control methods. One such method includes improving and developing rootstock resistance as a control method in the areas currently affected with ring nematode.

Resistance is seen as an effective and economical management tool for increasing the crop yield of both high and low value crops, in areas that are affected by high nematode densities. However, resistance in the case of grapevine has proved most effective against root-knot nematode (*Meloidogyne* spp.) (Loubser & Meyer, 1987; Roberts, 1992). Resistance, which is successfully and widely used today in crop production, is seen to pose much potential, thus requiring more effective utilisation (Starr *et al.,* 2002). However, both long-term and extensive effort is required for the development and identification of resistance (Starr *et al.,* 2002).

Until 1994 when the rootstock Guardian BY520-9 was introduced into commercial orchards, tolerance to *C. xenoplax*, and, in effect, PTSL, was unknown (Blenda *et al.,* 2007). In 1976, Zehr *et al*. recorded a reduction in the extent of tree loss to PTLS, due to the use of a relatively resistant rootstock. Lovell rootstock is recommended for use in areas that are infested with

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ring nematodes, as it is regarded as an important factor in avoiding the development of PTSL (Zehr *et al*., 1976). As yet, there has not been a rootstock that has been recorded as being resistant to *C. xenoplax* (Aballay *et al.,* 2009). Rootstock resistance to, or the tolerance of protection against, *C. xenoplax* is also seen as a viable management option (Pinkerton *et al*., 2005).

The use of rootstocks in viticulture has been implemented for over 150 years, as a protection against soil pests. Thus, over the years, research has focused on developing rootstocks that provide extensive and long-lasting resistance to grapevine pests (Zehr *et al*., 1976; Ferris *et al*., 2013). Currently, the stone fruit industry in South Africa is highly dependent on several commercially imported rootstocks. In many cases, however, the soil and local climatic conditions are not suited for the rootstock. As a result, continual improvement is required in developing the resistance of crops to, and their tolerance against, pests and diseases (Booi & Malan, 2013).

The objective of the current study was to determine the susceptibility of South African commercial grapevine rootstocks to *C. xenoplax*. The above was accomplished by means of determining the reproduction of ring nematode in pot trials in a glasshouse. Additionally, grapevine samples from a private laboratory were analysed with regard to different rootstocks and ring nematode numbers.

MATERIALS AND METHODS

EXTRACTION OF NEMATODES

The sugar centrifugation and flotation technique developed by Jenkins (1964) was used to extract the ring nematodes from the soil samples. To separate the organic matter, water was first used in the centrifugation method, followed by the use of a sugar solution. The nematodes were then separated and left in suspension from the soil particles, due to the soil's higher specific gravity. The rapid extraction of living and dead nematodes in a sample was made possible by the process used (Marais *et al.,* 2017). The suspension containing the nematodes

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was filtered through a 38-µm aperture sieve, and washed into a glass beaker for further processing. Exposure of the nematodes to the sugar solution was minimized, to decrease and prevent osmotic stress (Marais *et al.,* 2017).

SOURCE OF NEMATODE INOCULUM

The source of nematodes used for the experiment was obtained from pure cultures of *C. xenoplax* populations, which were maintained on the peach rootstock Atlas, which was maintained in the glasshouse at the ARC Infruitec-Nietvoorbij facility in Stellenbosch. The rootstocks used were grown in 5-L pots in a high-temperature-controlled glasshouse. A soil auger was used to take a 100 ml soil sample, from between the roots of each of the 15 plants. The nematodes collected from the samples were extracted using the sugar flotation technique (Jenkins, 1964), with the number of nematodes then being determined. From the 15 pots sampled, the pot with a nematode population closest to 1000 nematodes per 100 ml soil was selected. After removing the peach tree selected from its pot, all the soil adhering to the roots was shaken off and thoroughly mixed on a plastic bag. A glass beaker of 100 ml of infected soil was used as inoculum for the trial. After inoculation of the grapevine plants, ten 100-ml beakers of soil were kept separate and washed individually, to determine the actual number of nematodes used in the initial inoculum.

GRAPEVINE PREPARATION

Rooted grapevine cuttings approximately 4 months old were obtained from the Vititec nursery, Paarl in the Western Cape province of South Africa. In a glasshouse at Infruitec-Nietvoorbij, Stellenbosch, the plants were transplanted into 2-L plastic pots containing sterilised soil mixture, consisting of fine bark and river sand with a 2:1 ratio respectively, and with a pH of 6-6.5. The vines were left for a period of 2-3 weeks to settle, before inoculation was done with *C. xenoplax*.

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GLASSHOUSE TRIAL

A glasshouse trial was performed to evaluate the susceptibility of five commercially used grapevine rootstocks to *C. xenoplax*. The rootstocks used in the trial included Ramsey, 1130 Paulsen, 140 Ruggeri, 99 Richter and 110 Richter and sterilised soil which contained no plants was used as the control.

The trial was conducted throughout the months of December to May, during which time the temperature range was kept at 25-26°C. Once inoculated, the potted plants in the glasshouse were arranged in a completely randomised design. A total of 15 plants of each rootstock was used. The plants were inoculated 2 weeks after being transplanted. The grapevine plants were inoculated by means of adding 100 g of soil infected with *C. xenoplax* to each vine, after which the pots were watered to allow for the downward movement of *C. xenoplax* to the roots.

The plants were left in the glasshouse for a period of 6 months, after which they were removed from the pots. The soil and fine roots were placed in a plastic bag after shaking the plant to release soil from roots. *Criconemoides xenoplax* was extracted by means of processing 250 g of soil per sample, using the sugar flotation and centrifugation method (Jenkins, 1964).

ENUMERATION OF NEMATODES

The nematode suspension was left for 24 h to settle to the bottom of a 20-ml beaker, after which the top water was siphoned off to a volume of 20 ml. The nematodes were brought into suspension by means of bubbling air through the water, using a fish pump. The nematode densities for each sample were then determined by means of counting the nematodes present in the 2 × 1 ml suspension, using a Peter's slide with a light microscope at 40 times magnification. The average of the two readings for each sample was multiplied by 20 to obtain the nematode numbers present in the 20 ml of suspension. The reproduction factor (RF) was then calculated by means of dividing the final population of *C. xenoplax,* which was obtained by multiplying the total nematode count by the total volume of soil in the pot, by the initial

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population (Rf/Ri). The value was used to determine the resistance of the plants to ring nematode, with a low RF value, or a value of <1, indicating a poor host, or non-host, status. Conversely, a high RF value indicated a good host status.

ANALYSIS OF RESULTS FROM NEMLAB

Results from the grapevine soil samples, analysed during 2015 by a diagnostic nematode laboratory, Nemlab, near Klapmuts in the Western Cape province, were mined for data regarding rootstock and numbers of ring nematode. Only samples with complete information were used in the analysis. The nematodes were extracted from the soil samples using the same technique (Jenkins, 1964) as was used for all the extractions in the current study.

The results were analysed and classified according to the following criteria; zero for the complete absence of nematodes; low to mild when 1 to 200 ring nematodes per 250 ml of soil were found, and high to severely infected when 200 to 1000 ring nematodes were found.

STATISTICAL ANALYSIS

The results from the glasshouse experiment were analysed using STATISTICA (ver. 13.2). The mean number of ring nematodes obtained from the Nemlab samples and the glasshouse samples was analysed using analysis of variance (ANOVA), while the descriptive statistics, in the case of Excel, were used for the different rootstocks, and for the severity of infection recorded in the diagnostic samples.

RESULTS

GLASSHOUSE TRIAL

The results were analysed by means of a factorial ANOVA, with the main effects of rootstock and date showing a significant difference between the two trials conducted ($F_{(11, 158)} = 5.038$; $p < 0.005$), thus the results from the two trials could not be pooled and were analysed separately. A one-way ANOVA was used to analyse the differences between the mean numbers of *C. xenoplax* from the different grapevine rootstocks of the two trials.

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Trial 1 showed that the mean number of *C. xenoplax* on the rootstock 110 Richter (241 ± 51.66) differed significantly (p = 0.037) from Ramsey (32 ± 6.63) and soil only (p = 0.017), which served as the control. The rootstocks 1103 Paulsen (168 ± 49) and 140 Ruggeri (149 ± 28.32) did not differ (p > 0.05) from the mean number of *C. xenoplax* recorded on the other rootstocks tested. The rootstock 99 Richter (140 ± 32.72) had no significant difference recorded from the other grapevine rootstocks tested, except from for Ramsey (p = 0.04) (Fig. 3.1).

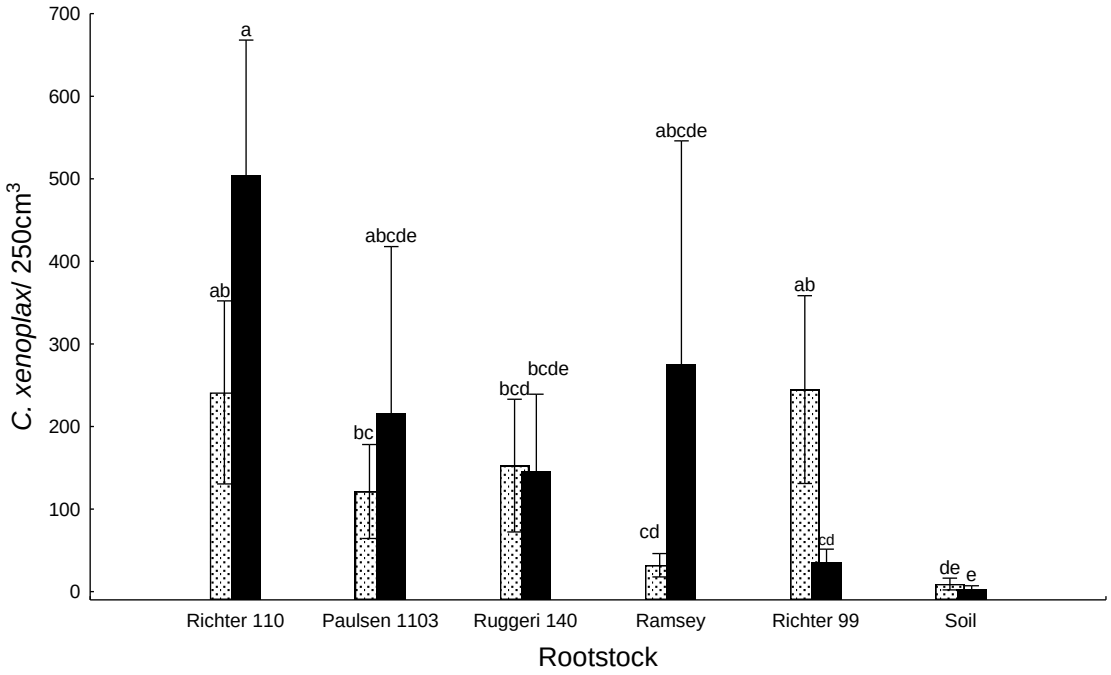

Figure 3.1. Results of the mean number of *Criconemoides xenoplax* numbers (Trial 1 = dotted bars; Trial 2 = black bars), recorded after 6 months, on the different rootstocks tested during a glasshouse experiment (one-way ANOVA $F_{(11, 158)} = 5.038$; $p < 0.005$). Different letters above the bars mean significant differences.

Results from trial 2 showed a significant difference between the mean number of *C. xenoplax* recorded from 110 Richter (504 ± 75.79), 140 Ruggeri (145 ± 43.77) (p = 0.02), and 99 Richter (35 ± 7.55) (p < 0.005). Rootstocks 1103 Paulsen (215 ± 94.5) and Ramsey (275 ± 105.41)

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did not indicate a significant difference ($p > 0.05$) in nematode numbers compared to the other grapevine rootstocks tested (Fig. 3.1). When comparing the results from the two trials, 99 Richter was the only rootstock that differed significantly ($p = 0.044$) in the mean number of *C. xenoplax* recorded.

REPRODUCTION FACTOR

The initial number of nematodes used for the inoculum showed that the initial population (R_i) was higher than had been expected, with 2300 nematodes per 100 ml soil. The RF values of all the rootstocks were relatively low, with 110 Richter having the highest RF value of 1.7. The other rootstocks, consisting of Ramsey, 1103 Paulsen, 140 Ruggeri, 99 Richter and soil, had RF values of 0.9, 0.8, 0.5 and 0.1 respectively, showing poor host status, as the RF values were below 1. Soil had little to no reproduction, with an RF value of 0.0 (Table 3.1).

TABLE 3.1. The reproduction factor (RF) calculated for the grapevine rootstocks tested in the glasshouse in both trials, showing the host status and the performance of the rootstock against *Criconemoides xenoplax*.

Rootstock	Clone	Genetic origin	Trial 1		Trial 2	
			No. of plants	RF value	No. of plants	RF value
110 Richter	RQ28	*V. berlandieri* × *V. rupestris*	15	0.2	14	1.7
1103 Paulsen	PS28	*V. berlandieri* × *V. rupestris*	15	0.1	15	0.8
140 Ruggeri	RU354	*V. berlandieri* × *V. rupestris*	15	0.1	15	0.5
Ramsey	SC18	*V. champini* Planch	15	0.0	15	0.9
99 Richter	RY13	*V. berlandieri* × *V. rupestris*	15	0.2	15	0.1
Soil	-	-	15	0.0	15	0.0

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ANALYSIS OF RESULTS FROM NEMLAB

The results from a total of 1430 grapevine soil samples from Nemlab, received during 2015, were used in the analysis. The overall results obtained from the grapevine rootstock from the vineyards indicated a significant difference between the mean populations of *C. xenoplax*, as observed on the different rootstocks ($F_{(3, 1430)} = 3.0077$, $p = 0.03$). The grapevine rootstock US 8-7 (561 ± 131.47) had the highest mean number of *C. xenoplax* present compared to the other rootstocks. However, no significant difference was recorded between US 8-7, 110 Richter (410 ± 52.68), and Ramsey (353 ± 19.62). The difference in the mean *C. xenoplax* population present on US 8-7 and 99 Richter was recorded as significant ($p = 0.038$) (Fig. 2).

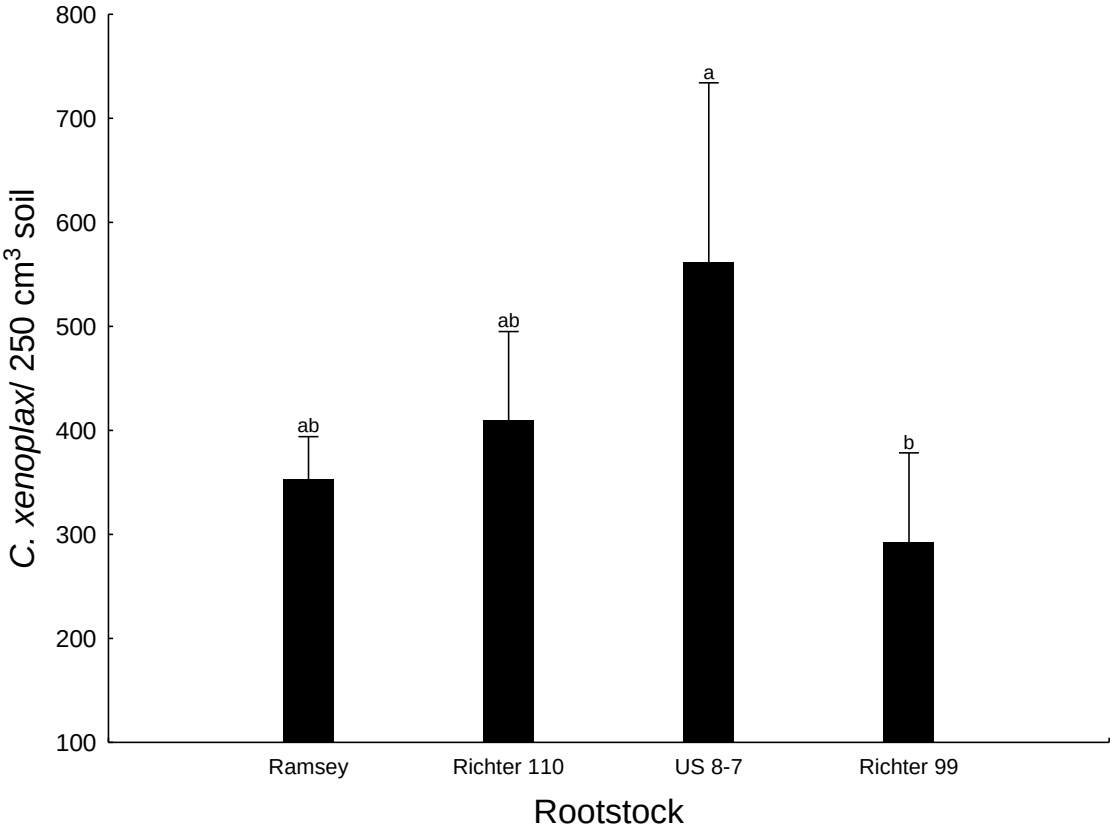

Figure 3.2. Mean *Criconemoides xenoplax* numbers recorded from Nemlab samples (n = 1430) received from January to February 2015 ($F_{3, 1430} = 3.0077$, $p = 0.03$). Different letters above the bars mean significant difference.

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A total of 948 samples of the grapevine rootstock Ramsey were analysed, of which 203 (21%) were found to have no ring nematode. A large percentage of the samples, 79% (745), were found to be infected. In terms of the low to mild infection rate (1 to 200 nematodes per 250 ml soil), a total of 352 samples (37%) were found to be infected with ring nematode, whereas 393 (41%) were found to be high to severely infected (200 to >1000 ring nematode per 250 ml soil). For the high to severely ring-nematode-infected soil, in 41% of the cases chemical treatment was suggested. Of the severely ring-nematode-infected samples, a total of 29 (7%) samples were found to contain >2000 nematodes per 250 ml soil, with the highest infection being found to be 6160 nematodes per 250 ml (Fig 3.3).

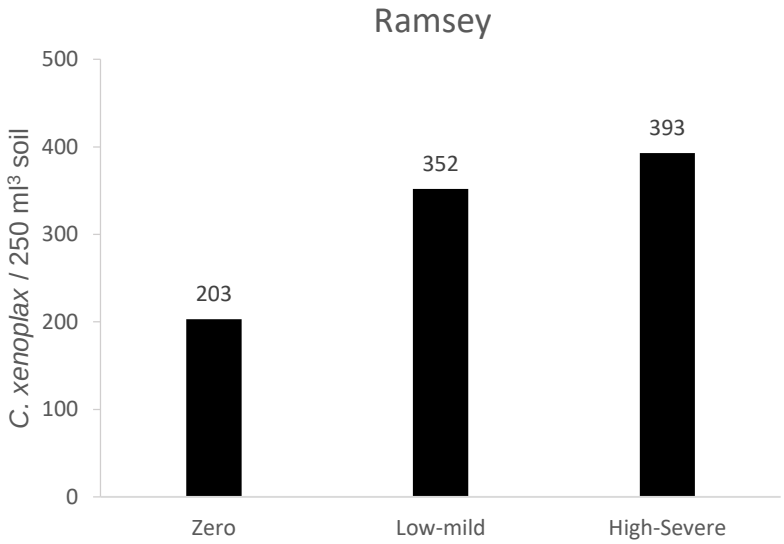

Figure 3.3. The total number of soil samples analysed from Nemlab, for 2015, for the grapevine rootstock, Ramsey. The results with regard to nematode numbers (per 250 ml^3 of soil) were classified as zero, with no *Criconemoides xenoplax*, low to mild with between 1 to 200 nematodes, and high to severe with nematodes between 200 and 1000.

In the case of 110 Richter, a total of 218 samples were analysed, of which 49 (22%) of the samples analysed had no ring nematodes recorded. However, the majority of the samples,

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169 (51%), were found to be infected. The infection rate of a total of 77 (35%) of the overall samples collected was classified as being low to mild, with 45% of the total number of plants being infected. Of the samples analysed, 92 (42%) were rated as high to severely infected, comprising a total of 54% of the infected samples. Of the samples collected, 26 had nematode numbers >1000, with the highest number of *C. xenoplax* being recorded from the samples collected as 4690 nematodes per 250 ml of soil (Fig 3.4).

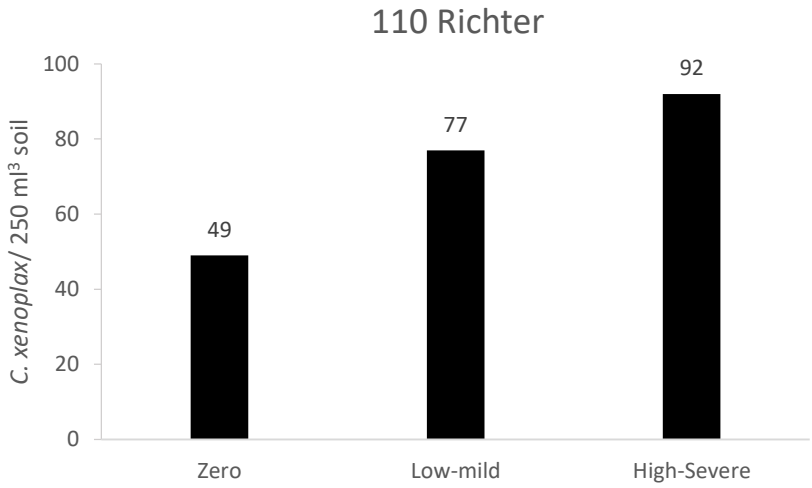

Figure 3.4. The total number of soil samples analysed from Nemlab, from January to December 2015, for the grapevine rootstock 110 Richter (n = 218). The results with regard to nematode numbers (per 250 ml^3 of soil) were classified as zero, with no *Criconemoides xenoplax*, low to mild with between 1 and 200 nematodes, and high to severe with the number of nematodes ranging from 200 to 1000.

For the grapevine rootstock US 8-7, a total of 53 samples were analysed. Of the samples analysed, 12 (23%) had no ring nematode present, whereas 41 were found to be infected. The infection rate of the total of 18 (34%) infected samples that had 1 to 200 nematodes present was classified as low to mild. Lastly, 23 (43%) of the samples were classified as having a high to severe infection rate. From the 23 samples, 11 had a ring nematode population

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greater than 1000, with 4550 ring nematodes per 250 ml of soil being recorded as the highest infestation of the rootstock concerned (Fig 3.5).

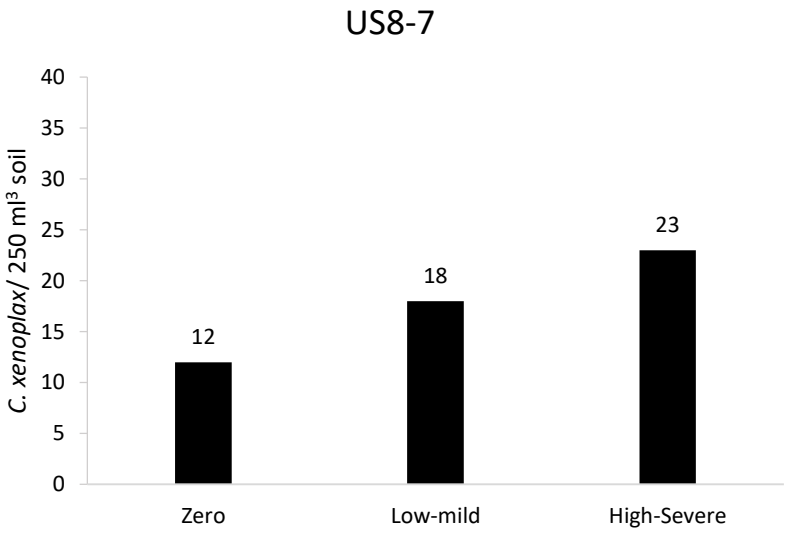

Figure 3.5. The total number of soil samples analysed from Nemlab, for January to December 2015, for the grapevine rootstock US 8-7 (n = 53). The results with regards to nematode numbers (per 250 ml^3 of soil) were classified as zero with no *Criconemoides xenoplax*, low to mild for between 1 and 200 nematodes, and high to severe for the number of nematodes ranging between 200 and 1000.

In the case of 99 Richter, a total of 216 samples were analysed. The ring nematode numbers were relatively similarly distributed between the three different classifications. Of the 216 samples analysed, 72 (33%) had no ring nematode present, with the infection rate of 74 (51%) of the infected samples being classified as low to mild, due to the presence of from 1 to 200 ring nematodes each, and, lastly, 70 (49%) of the total number of infected samples had a high to severe infestation of ring nematodes. A total of 22 (15%) of the infected samples had severe infestation, with numbers >1000, with the highest number recorded reaching 3980 nematodes per 250 ml soil (Fig 3.6).

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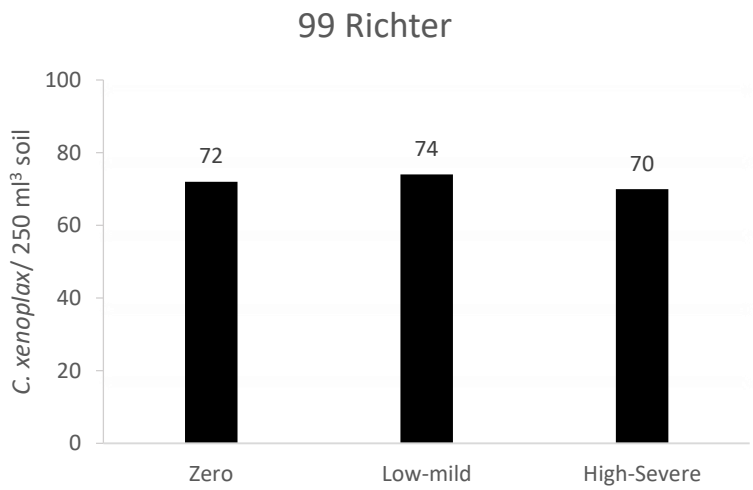

Figure 3.6. The total number of soil samples analysed from Nemlab, from January to December 2015, for the grapevine rootstock 99 Richter (n = 216). The results with regard to nematode numbers (per 250 ml^3 of soil) were classified as zero with no *Criconemoides xenoplax*, from low to mild with between 1 and 200 nematodes, and from high to severe with the number of nematodes present ranging between 200 and 1000.

In the case of Paulsen, only 11 samples were analysed, of which all the samples were classified as being highly to severely infected. All the samples had ring nematode numbers higher than 200 individuals per 250 ml soil, with the highest number being recorded as 2130 individuals.

DISCUSSION

Results from the two glasshouse trials differed significantly when comparing the final *C. xenoplax* numbers from the different grapevine rootstocks used in the glasshouse trial. The nematode numbers did not increase as drastically as was expected, when comparing the results obtained from other studies (Pinkerton *et al*., 2005; Schreiner *et al*., 2012). The reproduction factor (RF) values of the grapevine rootstocks studied was generally low (<1),

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except for that of 110 Richter. In a study carried out by Pinkerton *et al.* (2005), 110 Richter, which had the highest tolerance to *C. xenoplax*, was referred to as resistant. The rootstock 140 Ruggeri and 99 Richter were classified as susceptible, while 1103 Paulsen was regarded as being highly susceptible, as the populations increased by up to 20 times the initial population, resulting in RF values >6.9. In the 4-year study of Schreiner *et al*. (2012), 110 Richter, which had previously been recorded as having adequate tolerance, was found to have increasing *C. xenoplax* populations throughout the study.

The current discussion is based on the results of trial 2, as trial 1 had much lower *C. xenoplax* numbers, which could be ascribed to the overwatering of the plants concerned. At the end of the 6-month trial period, 110 Richter was recorded as having the highest mean number of *C. xenoplax* nematodes, and differed significantly compared to the other grapevine rootstocks tested. Ramsey had the second highest mean of nematodes recorded, followed by those of 1103 Paulsen, 140 Ruggeri and 99 Richter, respectively. No significant difference in nematode numbers was found between the different rootstocks, apart from for 110 Richter and 99 Richter.

Similarly, in 1987, Pieterse & Meyer recorded the grapevine rootstock, 110 Richter, as being the most susceptible to *C. xenoplax*. Population differences and increases observed between the studies concerned could be due to a number of factors, including soil composition, initial nematode population present (Pieterse & Meyer, 1987), root availability (Southey, 1992), soil moisture, and temperature (Ferris *et al.*, 2013). *Criconemoides xenoplax* reproduction on susceptible rootstocks was negatively affected by soil temperatures of 30°C and higher, with the populations being suppressed by up to 50% under such conditions (Ferris *et al.*, 2013). Nyczepir *et al*. (1987) recorded a faster increase in nematode populations in treatments that had relatively low initial populations, thus, to record notable increases in populations, the inoculation numbers should be low.

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The privately run nematode soil diagnostic laboratories in South Africa are an important source of information regarding the status, problems and trends of plant-parasitic nematodes in our local industries. The information generated from samples obtained from such laboratories is regarded as a confidential matter between the producer and the laboratory concerned. However, from the analysis of the overall results, valuable information can be gained by the industries involved, which can serve as a guide towards future research, and towards alerting those concerned of possible future problems with regard to specific plant-parasitic nematodes. The private laboratories do not always have administration systems that are readily accessible as sources of information, as the business concerned is geared to providing a service to individuals submitting samples for analysis. Trends and problems can be detected at an early stage by such laboratories, but, as the related results are not formally published, it is very difficult for both the industry and the producers concerned to act accordingly.

The results obtained in relation to the *C. xenoplax* numbers recorded from Nemlab on the grapevine rootstocks from vineyards revealed the presence of higher nematode numbers compared to those that were found in the glasshouse experiment. Nonetheless, the performance of the rootstocks in both the field and the glasshouse trials remained relatively similar, with 110 Richter having the highest *C. xenoplax* numbers recorded, and 99 Richter the lowest. No significant difference was observed in the *C. xenoplax* populations recorded on 110 Richter and Ramsey, although a significant difference was observed from those that were recorded in relation to 99 Richter. Overall, the rootstocks tested in the second glasshouse trial and those that were observed in the field trial were all found to be susceptible to *C. xenoplax*, with nematode numbers being highest for 110 Richter, and lowest for Ramsey and 99 Richter. The grapevine rootstocks analysed by Nemlab added up to a total of 1430, of which Ramsey (66.6%) made up the majority of the total number of samples. The rootstock, 110 Richter and 99 Richter, represented a total percentage of 15.2% and 15.1%, respectively, with US 8-7 forming 3.7%, and, lastly, 1103 Paulsen forming 0.8%. The number of severe cases were also seen to increase as the rootstock numbers increased, which could be seen in the case of

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Ramsey, which had the highest number of soil samples analysed, thus also resulting in a higher number of cases of rootstocks being severely infected by *C. xenoplax*. Of the severely ring-nematode- infected samples, a total of 30 samples were found to contain >2000 nematodes per 250 ml soil, with the highest infection rate being found to be 6160 nematodes per 250 ml of soil.

Plant resistance is relatively easy to identify compared to plant tolerance, as the former is usually described as having a monogenic trait (Starr & Bendezu, 2002). Tolerance is described as a plant's ability to combat parasitism by nematodes, without compromising the yield and the development of the plant (Roberts, 1992). However, environmental conditions could alter the tolerance of plants, as a result of which rootstock performance tends to vary between the different areas concerned (Pinkerton *et al.*, 2005).

The soil environment is a key factor in determining the distribution of roots within the soil, which is a result of the soil's physical, chemical and phytosanitary properties. Genetic entities, which differ in terms of the root distribution observed within a certain soil environment, refer to both scion and rootstock cultivars. The soil environment influences root distribution, while the genetic entity is a key factor in terms of root density (Southey, 1992). Southey (1992) found that no marked differences were present between the root distribution of the different rootstocks, Ramsey, 110 Richter, 99 Richter, 1103 Paulsen, and 140 Ruggeri. However, the root density did differ between the rootstocks tested, with Ramsey having the lowest root density, of 485 roots/m². The rootstock, 1103 Paulsen, 99 Richter, 140 Ruggeri and 110 Richter, had almost twice as many roots per m², with Paulsen having the highest density of 882 roots/m² (Southey, 1992). The *C. xenoplax* population differences observed on the rootstocks tested could, thus, have been the result of root density (Pinkerton *et al.*, 2005).

The control of *C. xenoplax* by means of such alternative methods as rootstock resistance is seen as an extremely viable method of keeping ring nematode populations below the threshold for the causation of damage. However, a resistant rootstock has, as yet, not been identified.

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Thus, it is important for further tests to be undertaken on rootstock susceptibility to *C. xenoplax*, to aid and improve the future control of the economically significant pest. The results obtained from the current study give an idea of the susceptibility of the different rootstocks analysed. However, understanding and determining the performance of the rootstocks against *C. xenoplax* under varying environmental conditions is likely to remain a daunting task, as there are many environmental factors that ultimately affect both the nematode, *C. xenoplax*, and the rootstock concerned.

For future research, a microplot study is recommended, in terms of which the relevant rootstocks should be exposed to more realistic environmental conditions, simulating those in the field. The conducting of such a study should allow for more accurate results regarding rootstock resistance and nematode population densities to be obtained.

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CHAPTER 4

CULTURING THE RING NEMATODE, *CRICONEMOIDES XENOPLAX*, USING ANNUAL HOSTS

ABSTRACT

The ring nematode, *Criconemoides xenoplax,* is an agriculturally important plant-parasitic nematode that has been recorded to have a wide host range, being found mainly on woody perennials, such as on its preferred hosts, grapevine and stone fruit. In addition, the nematode has also been observed on other plant species. The host status of five different annual plants, namely lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), carnations (*Dianthus caryophyllus*), mint (*Mentha*), sweet corn (*Zea mays var. saccharata*), and white clover (*Trifolium repens*) were evaluated in a glasshouse trial. The trial was carried out to determine whether *C. xenoplax* could be cultured *en masse* on alternative hosts, and to provide a faster and more successful culture method. The plants were inoculated with *C. xenoplax* 6 weeks after the replanting of seedlings, after which they were left for a period of 7 weeks to allow for nematode reproduction. The results showed that all the hosts tested during the trial did not sustain an increase in the nematode populations, as the RF (reproduction factor) values were all recorded below 1. Consequently, the annual plants tested were not considered suitable hosts for *C. xenoplax,* and they were not a viable option for the culturing of ring nematode populations for future experimental purposes. The current practice of using either grapevine or stone fruit should be employed, as they have been recorded to sustain substantial populations of nematodes in previous experiments.

Keywords: additional hosts, annuals, culture methods, *Criconemoides xenoplax*, ring nematode

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INTRODUCTION

Criconemoides xenoplax (Raski, 1952) Loof & De Grisse has been identified by numerous studies to be a key agent that is responsible for the occurrence of a disease complex referred to as peach tree short life (PTSL) in stone fruit, and for the global reduction in the growth of vineyards. Nyczepir *et al.* (1983) were the first researchers to prove the association of *C. xenoplax* with the PTSL syndrome. However, Raski & Radewald (1958) were the first researchers to reveal accurately the true parasitic nature of the ring nematode. The nematode is, thus, regarded as a significant pest in stone fruit orchards and vineyards worldwide, as it causes substantial crop loss. The nematode, *C. xenoplax*, has become an increasingly common problem in South Africa, with it being found to be the most common plant-parasitic nematode species found in the stone fruit orchards and vineyards concerned (Keetch & Heyns, 1982).

The ring nematode, Criconemoides xenoplax, is described as an ectoparasitic nematode that feeds exclusively on the roots of plants. Woody perennials are typically the preferred hosts of *C. xenoplax*, with the highest populations being recorded on apricot, plum, almond and grape (Seshadri, 1964). However, *C. xenoplax* and other members belonging to the genus Criconemoides have been reported to be associated with a wide variety of different plant species. The above includes, mint (Ingham & Merrifield, 1996), cherry (Melakeberhan *et al*., 1994), lettuce, carnation (Sher, 1959), tomato, clover (Zehr *et al*., 1990), and pine. Thus, rotation crops, vegetation native to an area, and leguminous plants have been shown to contribute to the persistence of *C. xenoplax* in the soil (Zehr et *al.,* 1990).

Feeding tends to occur along the length of the root, as well as at the root tip. Feeding of *C. xenoplax* was observed under laboratory conditions by Westcott & Hussey (1992). Once the roots of the host are located, probing occurs, until a suitable area for penetration is found. The establishment of feeding sites occurs at a specific cell, referred to as a ‘food cell’, in terms of which nutrients are withdrawn directly from the cytosol. Thus, nutrients flow directly through the opening formed in the plasma membrane (Hussey *et al*., 1992). The nematode feeds

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continuously at a specific root cortical cell for a period of 1 to 8 days, without damaging the cell itself. The observed feeding behaviour of *C. xenoplax* is considered to be more highly evolved than is the feeding behaviour of many other nematodes that feed on a number of cells within a short period of time (Westcott & Hussey, 1992).

The life cycle pattern of *C. xenoplax* is reported to be similar to that of other plant-parasitic nematodes. The cycle usually spans between 25 to 34 days, with the egg stage taking 11 to 13 days. The above is followed by the second, third and fourth larval stages, with, lastly, the preoviposition of the adults spanning a period of 2 to 3 days. Variation in the period of the different larval stages is observable, depending on the larval proximity to the host roots, with the result being the ability to feed, and to pass through the different developmental stages. Due to males being rare, reproduction is assumed to occur by means of parthenogenesis. Feeding is important for the development of the juvenile's oocyte maturation in females (Seshadri, 1964).

Optimum conditions, believed to be a combination of high temperature and high rainfall, are necessary for nematode reproduction. However, nematodes remain active and manage to persist in low numbers under unfavourable conditions (Nesmith *et al*., 1981; Pinochet & Cisneros, 1986). The relationship between host plants and parasites is largely affected by soil temperature. The rate of physiological processes, including the host plant response to infection growth, as well as increases in the nematode populations, are all affected by temperature (Griffin, 1969; Jatala & Russell, 1972; Thies & Fery, 1998; Ferris et *al*., 2013). The inactivity of most phytoparasitic nematodes is believed to be experienced between temperatures ranging from 5°C to 15°C, and from 30°C to 40°C. Optimum temperatures, therefore, tend to lie between 15°C and 30°C. For instance, in 1961, Lownsbery recorded an optimum temperature of 26°C for *C. xenoplax* reproduction. Extreme moisture settings also have a negative impact on nematode populations (Wallace, 1963). Soil moisture is suggested to be the main factor responsible for the size of nematode populations by several authors (Lawrence & Zehr, 1978; Nesmith *et al*., 1981).

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Culture techniques for plant-parasitic nematodes usually do not produce a large quantity of nematodes, with them generally being labour-intensive. As such, culture techniques are only limited to a small number of plant-parasitic nematodes (Walter *et al*., 1993). Edaphic conditions are important drivers affecting the population densities of nematodes in greenhouse cultures, together with root availability. However, nematode densities can be relatively lower than expected, with cultures, at times, failing (Walter *et al*., 1993). In 1959, Thomas collected reasonable numbers of *C. xenoplax* for inoculation from soil collected in the field. However, *C. xenoplax* populations that are currently used for inoculations in laboratory and field studies are cultured on suitable hosts. The numbers of *C. xenoplax* have been observed to increase, under greenhouse conditions, on the roots of Thompson's seedless grapes (Seshadri, 1964), as well as Nemaguard peach (Zehr *et al*., 1990).

The main aim of the current study was to test the host suitability of different annual hosts to provide a faster and more successful method of culturing *C. xenoplax* than at present under greenhouse conditions, thus enabling further research into the nematode.

MATERIALS AND METHODS

NEMATODE EXTRACTION

Nematodes were extracted using the sugar centrifugation and flotation method developed by Jenkins (1964), which relies on the specific gravity of nematodes to separate them from soil and organic debris. After centrifugation a suspension of organic matter with a specific gravity of <1 g cm−3 will stay and can be thrown out. The process allows for the rapid extraction of both living and dead nematodes from a sample (Marais *et al*., 2017). Afterwards, centrifugation in a sugar solution allows the nematodes to remain in suspension. The suspension containing the nematodes were then transferred to a 38-µm – aperture sieve and the nematodes retained on the sieve, were washed into a beaker containing distilled water for further analysis. Minimising the exposure of the nematodes to the sugar solution, to decrease and prevent osmotic stress, is important (Marais *et al*., 2017)

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SOURCE OF INOCULUM

The source of inoculum used in the experiment was obtained from soil containing monocultures of the ring nematode, maintained on the peach rootstock Atlas, which was grown in 5-L pots in a high-temperature-controlled glasshouse. A 100 ml soil sample was taken, using a soil auger, between the roots of each of the 15 plants. From each of the samples collected, the nematodes were extracted by using the sugar flotation technique, whereupon the number of nematodes was counted. From the 15 pots sampled, the pot with the number of nematodes closest to 2000 nematodes / 100 ml soil was selected. Upon removing the peach tree from the pot, all the soil was shaken from the roots and thoroughly mixed. A 100-ml glass beaker was used as inoculum for the trial. Ten 100-ml beakers of soil were kept separately and washed individually, to determine the actual number of nematodes used as the initial inoculum.

GLASSHOUSE TRIAL

A glasshouse trial was performed to evaluate the host suitability, and possible culture varieties, for the rearing of *C. xenoplax* for purposes of mass production. Five different annual plants were used in the trial, including lettuce (*Lactuca sativa*) var. Great Lakes, tomato (*Lycopersicon esculentum*) var. Moneymaker, carnations (*Dianthus caryophyllus*), mint (*Mentha*), sweet corn (*Zea mays* var*. saccharata*), and white clover (*Trifolium repens*). All the plants were grown from seed in steam-sterilised soil for a period of 7 weeks.

The trial was conducted in a glasshouse at Infruitec-Nietvoorbij, Stellenbosch, Western Cape province of South Africa, where the temperature range was kept at 25-26°C, with a humidity of 50%. After 6 to 7 weeks, the plants were transferred to 2-L pots, containing a sterilised soil mixture, consisting of fine bark and river sand, in a 2:1 ratio respectively, and with a pH of 6 to 6.5. Once potted, the plants were arranged in a completely randomised design in the glasshouse. A total of 15 plants per host was used, and pots with soil only were included as the control in the trial. Plants were inoculated two weeks after transplanting, with the known number of nematodes being approximately 855 individuals per plant. The plant hosts were

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inoculated by means of adding 100 g of soil infected with *C. xenoplax* to each plant. The pots were then watered, to allow for the downward movement of *C. xenoplax* to the roots.

After 7 weeks, the plants were removed from the pots, and the soil and fine roots were placed in a plastic bag, by means of shaking the plant to release soil from the roots and ring nematodes were extracted as before

NEMATODE ENUMERATION

Nematode densities for each sample were then determined by means of counting the number of nematodes present in 2 × 1 ml suspension using a Peter's slide under × 40 magnification, employing a light microscope. The average of the two readings for each sample was recorded and multiplied by 20 to obtain the nematode numbers present in 20 ml suspension. The reproduction factor (RF) was then calculated by means of dividing the final population of *C. xenoplax* by the initial population (Rf/Ri). The value obtained was used to determine the reproduction of ring nematode, with a low RF value, or a value of 1 or 0, indicating poor host or non-host status. Conversely, a high RF value indicated a good host status.

STATISTICAL ANALYSIS

The results from the glasshouse experiment were analysed using STATISTICA (ver. 13.2). The mean number of ring nematodes in the glasshouse samples was analysed using an analysis of variance (ANOVA) to account for any differences observed in the number of *C. xenoplax* present between the different hosts tested.

RESULTS

There was an overall significant difference recorded in the mean number of *C. xenoplax* found between each of the hosts tested during the experiment ($F_{(6, 93)} = 6.2356$, $\rho < 0.005$). Slight variation was observed within the different hosts tested, being lettuce (13 ± 1.76), tomato (31 ± 6.68), mint (9 ± 3.09), sweet corn (13 ± 3.03), clover (11 ± 3.29), and carnation (9 ± 2.87). The results showed no significant difference ($\rho > 0.05$), between the *C. xenoplax* numbers recorded on the different hosts. However, lettuce ($\rho = 0.01$) and tomato ($\rho = 0.01$) did differ

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significantly from soil only (3 ± 2.06), which was used as the control for the trial. Tomato had the most variation in *C. xenoplax* numbers from one plant to another, compared to the other host plants tested (Fig. 4.1).

The reproduction factor of all the annual hosts tested was less than 1. Tomato had the highest RF value (0.3) compared to lettuce, mint, sweet corn, clover, and carnation, which all had an RF value of 0.1. Soil had little to no reproduction, with an RF value of 0.0 (Table 4.1).

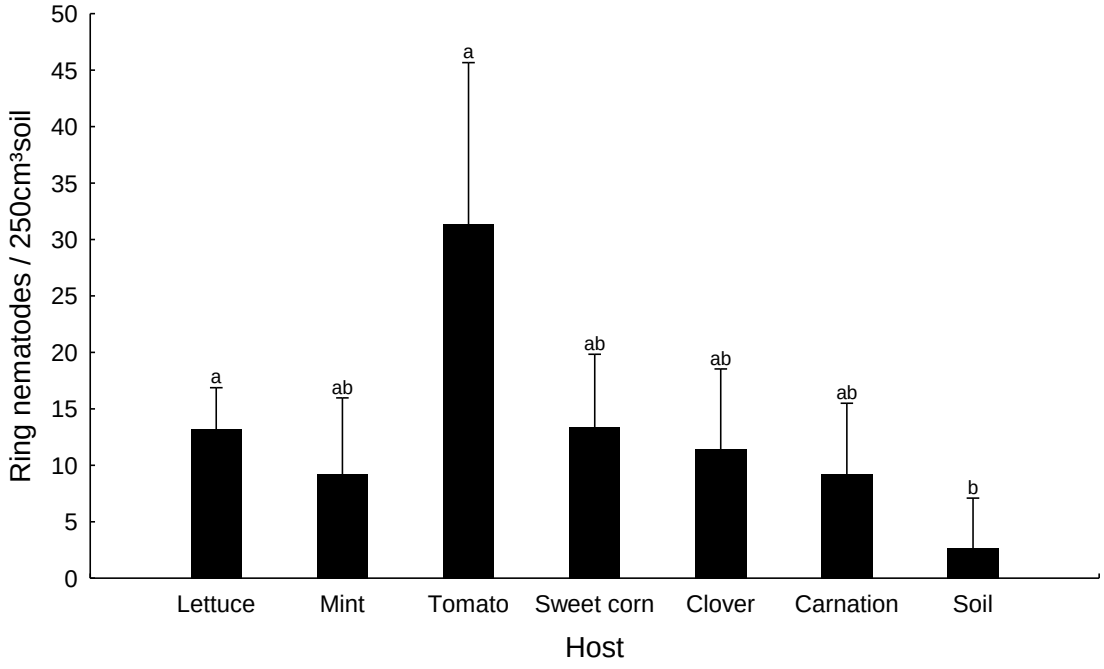

Figure 4.1. Results of the mean number of *Criconemoides xenoplax*, recorded after 6 months, on the different annual hosts tested during a glasshouse experiment ($F_{(6, 93)} = 6.2356$, $\rho < 0.005$).The same letter on the bar means no significant difference.

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Table 4.1. Reproduction factor calculated for the different hosts tested in the glasshouse trial, showing host status and performance of the hosts against *Criconemoides xenoplax*.

Host	Variety	Number of replicates	RF value
Lettuce	*Lactuca sativa*	16	0.1
Mint	*Mentha* × *piperita*	13	0.1
Tomato	*Lycopersicon esculentum*	15	0.3
Sweet corn	*Zea mays* var. saccharata	15	0.1
Clover	*Trifolium repens*	14	0.1
Carnations	*Dianthus caryophyllus*	13	0.1
Soil	-	15	0.0

DISCUSSION

The plants tested during the glasshouse trial were selected, as the presence of *C. xenoplax* was reported on the different annual plants (Westcott & Hussey, 1992; Core, 2001). All of the annual hosts tested in the glasshouse trial did not encourage nematode reproduction. Thus, from the results obtained, none of the plants tested are suitable hosts for *C. xenoplax* reproduction. Tomato proved to be the best host when compared to the others. However, the nematode numbers were still very low when compared to the other studies conducted. In Kruger *et al.*'s (2015) study, using tomato as a control, > 300 *C. xenoplax* individuals were recovered after 12 weeks.

A study, carried out in 1992 by Westcott & Hussey, tested *C. xenoplax* feeding behaviour in monoxenic cultures. A slight difference in *C. xenoplax* behaviour was recorded on the hosts tested. Activity of the nematodes on the crimson clover was recorded for a period of 15 to 20 weeks after nematode establishment, with the deposition of eggs also being recorded. The accumulation of second-stage juveniles did not occur in the culture, demonstrating that the juveniles advanced to the following phase. The development of second-stage juveniles ceased on the tomato and carnation root cultures, resulting in the accumulation of second-

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stage juveniles, thus indicating that the feeding of juveniles on the roots did not occur (Westcott & Hussey, 1992).

The plant hosts tested during the study carried out by Westcott & Hussey (1992) were all reported as being suitable hosts for ring nematode, as the ring nematode populations concerned increased from between 6.6 to 120 times on the various hosts tested. However, even though tomato and carnation were recorded as having a good host status for *C. xenoplax* in monoxenic cultures, the failed development of second-stage juveniles indicated that they did not feed on the available roots. The event recorded has not yet been observed in the soil environment (Westcott & Hussey, 1992). The results of the study carried out by Westcott & Hussey (1992) could indicate why the size of *C. xenoplax* populations did not increase in the study conducted.

The feeding behaviour of *C. xenoplax* is described as ectoparasitic. However, it is recorded as being different from other ectoparasitic nematodes, as feeding occurs at a specific cell for a period of 1 to 8 days. Thus, the feeding behaviour of *C. xenoplax* is seen as being highly advanced when compared to that of nematodes that feed at one cell for a short period of time (Westcott & Hussey, 1992). The movement of *C. xenoplax* was previously recorded to resemble that of an earthworm, with the body shortening and elongating. However, during the study, the nematodes moved similarly to other soilborne nematodes, moving in a serpent-like manner (Thomas, 1959; Westcott & Hussey, 1992).

The need to culture *C. xenoplax* is largely driven by the need to enable researchers to experiment on alternative methods of control, as well as to gain an enhanced understanding of the nematode-plant interaction. The control of *C. xenoplax* is considered to be a challenging task compared to that of other plant-parasitic nematodes, due to the thickness of its cuticle (Kruger *et al.*, 2015), and the depth at which the nematode occurs in the soil. It is, therefore, important to conduct continued and more in-depth research into the nematode to gain more knowledge on its feeding behaviour and preferred host status. To conduct future studies, a constant and reliable supply of *C. xenoplax* cultures should be available. To achieve this,

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however, is a constant struggle, as the nematode is notoriously difficult to culture and to maintain in high numbers for experimental use.

Previous studies made use of ring nematodes cultured on peach and grapevine hosts to carry out various trials (Santo & Bolander, 1977; Westcott & Hussey, 1992; Nyczepir *et al.*, 2009). Perennial hosts are recorded to be the preferred hosts of the ring nematode, and exceptionally high *C. xenoplax* populations have been observed in the field and in greenhouse studies (Mojtahedi & Lownsbery, 1976; Nycezepir, 1985). On investigating several herbaceous plants to determine their host status for *C. xenoplax*, Zehr *et al.* (1986) reported that nematode populations decreased on the majority of the hosts tested. Although some plants could maintain a relatively low population of *C. xenoplax*, they were not regarded as suitable hosts (Zehr *et al.*, 1986).

In relation to the analysis of soil from the grapevine samples analysed by a private laboratory, Nemlab, much higher numbers of nematodes were recorded from vineyards in comparison to the numbers that were obtained from stone fruit samples (Chapter 2). Thus, it should be noted that, for the mass culture of ring nematode as inoculum for further studies, grapevine could be regarded as a better option than stone fruit. However, further research should be carried out to test grapevine and stone fruit performance in a glasshouse, with regards to nematode reproduction and population increase over a period of time. Other hosts, such as pecan trees (Kleynhans, 1986; Nyczepir & Wood, 2008) and walnut trees (Ciancio & Grasso, 1998; Lownsbery *et al.*, 1978), could also be included in the trials, as they have also been recorded as being a good host for *C. xenoplax*.

For future research into ring nematode culture methods, it is important to note that annuals are not suitable hosts for the mass culturing of *C. xenoplax,* as there was little to no reproduction observed over the weeks during which the trials concerned took place. Consequently, perennial hosts should be further studied and used to improve the culture methods of the nematode involved. Experiments should focus on the ring nematode life cycle and on feeding habits to establish the most favourable conditions required for nematode

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reproduction such as, temperature, soil type, soil moisture conditions, rootstock and other factors that might affect plant-nematode interaction.

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CHAPTER 5

GENERAL CONCLUSION

From the data collected and analysed during the current study, it is apparent that the ring nematode, *Criconemoides xenoplax,* has become an increasingly common and significant pest in the stone fruit and grape industries in South Africa. The phenomenon has also been recorded by several different nematode diagnostic laboratories in the area. Differences in ring nematode densities and distribution were observed between the different production areas sampled, as well as between the different preferred hosts tested. *Criconemoides xenoplax* density and occurrence was recorded to be highest on grapevines, followed by plums.

The soil samples, collected from the respective stone fruit and grapevine production areas in the Western and Northern Cape regions, recorded 100% occurrence of *C. xenoplax*. However, after analysing the morphology and the ITS region of the ring nematodes collected from the different regions, all ring nematodes isolated were identified as *C. xenoplax*. However, an unknown ring nematode species was found on pear in the Grabouw area. The ring nematode species differed with 82% when blasted on GenBank, compared to *C. xenoplax*, with it being only morphologically shorter in body length. The ring nematode numbers varied from as few as 20 nematodes to over 3000 nematodes per 250 ml of soil, validating its pest status as economically important. The distribution of the ring nematode in South Africa follows trends similar to those observed worldwide, in areas experiencing problems with the pest. Thus, the need to develop sound management options to control the nematode pest in affected production areas is crucial for sustaining the health of the stone fruit and grape industry in South Africa.

The increased problems associated with *C. xenoplax* are of great concern to the different industries, as the control of the nematode has proven to be a continuous task. Nematicides previously employed for ring nematode control have been removed from the market, or their use has been severely restricted. The recent move of the grape and fruit industry to more

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sustainable and green agricultural practices has led to the research and implementation of alternative control measures to fill the gap left by the restricted use of chemicals. However, no suitable replacement has yet been found to manage ring nematode to acceptable levels in infested fields. The above will have implications for the drive to the sustainability and health of agricultural soil, as chemical control through soil application is currently the only control measure available.

The use of resistant rootstocks as an alternative method of control has become a favourable and common practice for the control of ring nematode infestation in orchards and vineyards in South Africa. However, the adoption of such an alternative approach is not as effective as is the use of chemicals to control ring nematode populations. The data obtained from evaluating six commercial rootstocks in a glasshouse for *C. xenoplax* susceptibility, and additional data from a diagnostic nematode laboratory, indicate that there was a significant difference in *C. xenoplax* numbers between the grapevine rootstocks assessed. The rootstock 110 Richter was recorded to have the highest susceptibility for *C. xenoplax*, as the highest number of ring nematodes were recorded in the soil, compared to the other rootstocks.

Richter 99 was least susceptible to *C. xenoplax*, with the above being recorded in both the glasshouse trial, and in samples from Nemlab. Ring nematode numbers were generally higher in the soil samples from Nemlab, compared to those obtained in the glasshouse trial. To further understand rootstock resistance, glasshouse trials could be replaced by microplot studies, to ensure that the rootstocks are exposed to more realistic environmental conditions than those to which they would be exposed in the field. Regarding the rootstock resistance to *C. xenoplax*, microplot studies would provide a more accurate conclusion, as both the nematode and rootstock performance would, consequently, be studied under natural field conditions. Such a study will help in gaining an understanding of the effects that the multitude of biotic and abiotic interactions would have on nematode populations in the field.

A screening programme for new and current grapevine rootstocks should also, as a matter of priority, be implemented against ring nematode. The practice has, for many years, been

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implemented for stone fruit. Ring nematodes also tend to occur in higher numbers in warmer areas, such as in the Northern Cape regions of Kakamas, Blouputs and Augrabies, and in the Western Cape region of Worcester, with nematode numbers reaching from 800 to 2100 nematodes per 250 ml of soil in the respective areas. Thus, climate change might lead to benefiting ring nematode reproduction in certain production areas.

Culturing *C. xenoplax en masse* has proven to be a challenging task, as the populations concerned did not increase in number on the various annual hosts tested. The results obtained during the study did not correlate with previous studies testing the host suitability of the specific annuals used. Future studies should continue to rear such monoxenic cultures of *C. xenoplax* as grapevine or stone fruit, with the preferred host being grapevine, as the latter have been shown in the current study to sustain high numbers of ring nematodes. No other hosts are, thus, currently suitable for the culturing of the nematode concerned. Cover cropping in orchards and vineyards should also be investigated, as some herbaceous plants have been noted to maintain ring nematode populations, and sometimes to act as, a good host for the nematode in the field.

Only one year of Nemlab data have been analysed in the current study. Data obtained from Nemlab and other private companies over a number of years could contribute to the development of a large-scale database to help researchers gain enhanced insight into the distribution and effect of *C. xenoplax* in grapevine and stone fruit orchards. The data collected could, furthermore, be utilised to show the trends in *C. xenoplax* density and distribution over the years. Thus, such environmental factors as temperature, rainfall, soil moisture, and others could also be studied longitudinally, to assess which factors play an important role in ring nematode increases and damage over several years.

To conclude, the ring nematode, *C. xenoplax,* is a very challenging nematode with which to work, and more research needs to be carried out on it in South Africa. Doing so would enhance the understanding and amount of knowledge pertaining to the biology of such an economically important pest.